Universidade de Aveiro
2011

Departamento de Ambiente
e Ordenamento

Iryna Lanko

Anaerobic acidification of cheese-whey in the MBBR reactor

Universidade de Aveiro

2011

Departamento de Ambiente

e Ordenamento

Iryna Lanko

Anaerobic acidification of cheese-whey in the MBBR reactor

Master thesis is presented to the University of Aveiro (UA) to fulfil the requirements for the Master degree in Environmental Studies (Joint European Master Program in Environmental Studies – JEMES). It was done under the scientific supervision of Doctor Maria Isabel Aparicio Paulo Fernandes Capela, Professor of the Department of Environment and Planning of the UA and co-supervision of Doctor Kerstin Kuchta, Professor at the Institute of Environmental Technology and Energy Economics of Technical University of Hamburg-Harburg (TUHH).

Financial support of the European Commission through the Erasmus Mundus Program, for the Master scholarship.

jury

President	Prof. Dr. Ana Isabel Couto Neto da Silva Miranda, Departament of Environment and Planning of University of Aveiro
External examiner	Prof. Dr. Cheng Chia-Yau, Department of Engineering of University of Porto
Supervisor	Prof. Dr. Maria Isabel Aparicio Paulo Fernandes Capela, Departament of Environment and Planning of University of Aveiro

acknowledgements

I am very thankful to my supervisor Prof. Maria Isabel Aparicio Paulo Fernandes Capela for her guidance and support from the initial to the final stage of the work what enabled me to develop an understanding of the subject and writing the thesis. I am also very thankful to my co-supervisor Prof. Kerstin Kuchta for important suggestions and ideas which made my work more full and sufficient.

I am grateful to the PhD student Flavio Castro Silva for his assistance during laboratory experiments and useful advice, Joao Sousa for his help in processing and obtaining the results and technical support. I am also thankful to all members of "AGUA group" and JEMES coordinators and students for moral support and motivation.

I would also like to thank the JEMES consortium for the unforgettable program and the European Commission for the scholarship.

keywords anaerobic acidification, VFA production, polymer production (PHAs), COD removal, waste treatment valorisation, MBBR reactor technology, cheese-whey

abstract In this study cheese-whey conversion into VFAs as a source for biopolymers production was investigated. Cheese-whey was chosen due to its high organic content being a by-product from the cheese production factory, as a part of valorisation methodology for industrial waste streams. Cheese-whey acidification process was used as an alternative to the waste treatment technologies.

To study the acidification of cheese-whey, a set of experiments was carried out to produce short-chain volatile fatty acids (VFAs), in order to find out its ratio to the total chemical oxygen demand (tCOD) of feed present in the reactor. The proportional amounts of Acetic, Propionic and i-Butyric acids towards the rest of the VFAs were also important in order to evaluate the MBBR efficiency for different operational parameters such as hydraulic retention time (HRT), alkalinity and organic load rate applied (OLR). To fulfil these goals the mass balances of the system were performed.

The maximum production rates of Acetic, Propionic and i-Butyric acids associated with simultaneous changes in OLR and alkalinity at a constant HRT of 12 h, were investigated (70% and 65% of total VFAs produced – at Phases 0 and 4, respectively). The degree of acidification of cheese-whey to the short-chain VFAs was about 33% and 27% of the influent COD concentration, at Phases 0 and 4, respectively.

The optimum operational conditions under study where the maximum production rates of Acetic, Propionic and i-Butyric acids occurred were at an alkalinity of 3.6 $gCaCO_3/L$ and an OLR = 35 gCOD/L*d (Phase 4). At this optimum conditions for acids production, the average rate of COD removal was equal to 20% and the rate of methane production was equal to zero.

Table of contents

List of figures 6
List of tables 8
List of abbreviations 9
1 Introduction 11
2 Bibliographic review 14
2.1 Acidification process 14
2.2 Moving bed bioreactor 14
2.3 Production of polyhydroxyalkanoates (PHAs) 15
3 Objective of the study 17
4 Methodology 18
4.1 The substarte characterisation 18
4.2 The experimental set-up 20
5 Experimental methods 25
5.1 COD test 25
5.2 TSS and VSS analyses 26
5.3 Alkalinity test 26
5.4 VFA test 27
5.5 Mass balance analysis 27
6 Results and discussion 32
6.1 pH, alkalinity and VFA evolution 33
6.2 Organic matter degradation 37
6.3 Biogas production 46
6.4 Biomass evaluation 51
6.4.1 Quantification of biomass 51
6.4.2 Microscopic evaluation 56
6.5 The mass balance of the system 60
7 Conclusion 71
8 Suggestions for future work 73
9 References 74
10 Appendix 76

List of figures

Fig. 4.1 – The scheme of experimental set-up and location of sampling points;

Fig. 4.2 – The set-up in the real conditions;

Fig. 4.3 – A view of carrier "Biofilm 9";

Fig. 5.1 – The scheme of organic matter degradation;

Fig. 5.2 – The chromatograph of the 11th day of sampling;

Fig. 6.1 – average values of pH, alk_{out}, alk_{in} & VFA data vs. time during the whole of experiment;

Fig. 6.2 – pH, alk_{out}, alk_{in} & VFA data vs. time during the 1st (left, up) and 2nd (right, up) the 3rd (left, down) and 4th (right, down) phases of experimental time;

Fig. 6.3 – The correlation of VFAs amount, COD_{in} and COD_{out} data vs. time;

Fig. 6.4 – The relation between VFAs and COD_{in}, VFAs and COD_{out}, COD_{out} and COD_{in} vs. time;

Fig. 6.5 – The distribution of VFAs, mgCOD/L;

Fig. 6.6 – The distribution of VFAs, %;

Fig. 6.7 – The VFAs' distribution during Phase A and B of Phase 2;

Fig. 6.8. – The VFAs' distribution inside the reactor;

Fig. 6.9 – The total amount of biogas and its main components (CO_2 and CH_4);

Fig. 6.10 – The main biogas components (CO_2 and CH_4) as 100% distribution;

Fig. 6.11 – CH_4 distribution during the 1st time period of experiment;

Fig. 6.12 – CH_4 distribution during the 2nd time period of experiment;

Fig. 6.13 – CH_4 distribution during the 3rd time period of experiment;

Fig. 6.14 – CH_4 distribution during the 4th time period of experiment;

Fig. 6.15 – The concentration of TSS & VSS in the MBBR reactor and clarifier vs. time;

Fig. 6.16 – TSS and VSS concentration during the 1st (left) and 2nd (right) phases of experiment;

Fig. 6.17 – TSS and VSS concentration during the 3rd (left) and 4th (right) phases of experiment;

Fig. 6.18 – The photos of the content of MBBR reactor at the OLR = 33 gCOD/L*d, magnified in 10 and 100 times, respectively;

Fig. 6.19 – The photos of the content of MBBR reactor (upper photos – sample of mixed liquor suspended solids; middle photos – sample taken from the outer side of carriers; lower photos – sample taken from the inner side of carriers) after reactor

opening (when the OLR = 66 gCOD/L*d), magnified in 10 and 100 times, respectively;
Fig. 6.20 – The photos of the content of MBBR reactor at the OLR = 35 gCOD/L*d, magnified in 10 and 100 times, respectively;
Fig. 6.21 – The data from the 2nd step of mass balance scheme;
Fig. 6.22 – The relation between the COD_{in} and COD_{sum1} (2nd step);
Fig. 6.23 – The data from the 3rd step of mass balance scheme;
Fig. 6.24 – The relation between the COD_{in} and COD_{sum2} (3rd step);
Fig. 6.25 – The data from the 4th step of mass balance scheme;
Fig. 6.26 – The data of 4th step of mass balance including the data from Phase 0 (previous study) and Phases A-B (split of Phase 2);
Fig. 6.27 – The relation between the COD_{in} and COD_{sum3} including the data from Phase 0 and Phases A-B (4th step).

List of tables

Tab. 4.1 – Composition of the cheese-whey broth medium [9];

Tab. 4.2 – The main characteristics of cheese-whey used in the experiments;

Tab. 4.3 – The operational parameters and phases of the experiments;

Tab. 4.4 – The composition of MBBR feed;

Tab. 4.5 – The content of micro- and macronutrients solutions;

Tab. 5.1 – The values of coefficients «m», «b» and other parameters and limits of VFA chromatograms;

Tab. 5.2 – The thOD calculated for each VFA, g;

Tab. 6.1 – The schedule of experimental frameworks;

Tab. 6.2 – The real OLRs according to the experimental data obtained;

Tab. 6.3 – Phases and parameters of experiment;

Tab. 6.4 – The COD removal rate at different periods of experiment;

Tab. 6.5 – The Acetic, Propionic and i-Butyric acids' yield production;

Tab. 6.6 – The COD_{VFA} ratios to COD;

Tab. 6.7 – The average values of total biogas and CH_4 amounts;

Tab. 6.8 – The average values of TSS and VSS data according to Figure 6.14;

Tab. 6.9 – The average values of TSS and VSS data;

Tab. 6.10 – The ratios of TSS and VSS in the clarifier (CLAR) and MBBR reactor;

Tab. 6.11 – The mass balance of the system;

Tab. 6.12 – Comparison results between Phase 0 (previous set of experiments) and Phases 1-4 (current experiment) [15].

List of abbreviations

S – substrate, referred as COD units, gCOD/L;

X – sludge biomass, referred as VSS units, gVSS/L;

MBBR – moving bed bioreactor;

CLAR – clarifier;

SSA – specific surface area, m^2/m^3;

PHA – polyhydroxyalkanoate;

HRT – hydraulic retention time, h;

OLR – organic load rate, gCOD/L*d;

SS – suspended solids, g/L;

TSS – total suspended solids, g/L;

VSS – volatile suspended solids, g/L;

BOD – biological oxygen demand, mg/L;

alk_{in} – alkalinity of feeding solution, $gCaCO_3/L$ ($gNaHCO_3/L$);

alk_{out} – alkalinity of effluent from the MBBR reactor or clarifier, $gCaCO_3/L$ ($gNaHCO_3/L$);

sCOD – soluable chemical oxygen demand, mg/L;

tCOD – total chemical oxygen demand, mg/L;

thOD – theoretical oxygen demand, mg/L;

STP – standard temperature and pressure;

VFA – volatile fatty acid, mgCOD/L;

FID – flame ionisation detector;

TCD – thermal conductivity detector;

COD_{in} – COD parameter of the feeding solution obtained experimentally, mg/L;

COD_r – COD parameter of content inside the MBBR reactor obtained experimentally, mg/L;

COD_{CH4} – COD parameter of methane constituent of biogas calculated by Equation 5.1, mg/L;

COD_{bio_dif} – COD parameter of biomass inside the MBBR reactor calculated by subtracting from COD_{in} the sum of COD_r and COD_{CH4}, mg/L;

COD_{bio_calc} – COD parameter of biomass inside the MBBR reactor calculated by Equation 5.2, mg/L;

COD_{bio} – COD parameter of biomass inside the clarifier calculated by Equation 5.2,

mg/L;

COD_{out} – COD parameter of content inside the clarifier obtained experimentally, mg/L;

COD_{VFA} – COD parameter of VFAs calculated by Equation 5.3, mg/L;

COD_{other} – COD parameter of unidetified constituents of final fermentation products calculated by subtracting COD_{VFA} from COD_{out}, mg/L;

COD_{sum1} – COD parameter which consists of $COD_r + COD_{bio\_calc} + COD_{CH4}$, mg/L;

COD_{sum2} – COD parameter which consists of $COD_{out} + COD_{bio} + COD_{CH4}$, mg/L;

COD_{sum3} – COD parameter which consists of $COD_{VFA} + COD_{other} + COD_{bio} + COD_{CH4}$, mg/L.

1 Introduction

Awareness concerning the environment and the use of clean technologies is growing worldwide. As a consequence, research on biodegradability and use of renewable resources for industrial processes has been intensive in the last years. Within the context of environmental biotechnology and commodity production biological-derived polyesters represent a potentially sustainable replacement to fossil fuel based on thermoplastics.

During recent years a variety of biopolymers have become available for use in many applications that are not only compatible with human lifestyle but also are friendly to the environment. Biopolymers are superior to petrochemical-derived polymers in several aspects that include biocompatibility, biodegradability and both environmental and human compatibility. Biopolymers can be classified according to the monomers that constitute them, which include various polysaccharides, polyamides (proteins and poly-γ-glutamic acid (γ-PGA)), nucleic acids (DNA and RNA), polyesters (polyhydroxyalkanoates, PHAs), polyphosphates and polyisoprenoids (natural rubber). Nowadays, some of these biopolymers are produced by bacterial fermentation and used commercially in a wide range of applications such as food, pharmaceuticals, plastics and agriculture.

In general, microbial synthesis of PHAs occurs as an energy reserve strategy when the cells are subjected to an excess of carbon source and deprived of one of the key nutrients for growth (N, P, etc.).

The structure and chemical composition of biopolymers are rather complex, which makes their chemical synthesis both inefficient and expensive. Therefore, the development of biotechnological processes is an inevitable route towards the economic production of biopolymers. Not only refined carbohydrates but also agricultural and dairy by-products can be used as substrates for the production of these biopolymers by fermentation processes [1].

The intermediary compounds produced during anaerobic biodegradation – volatile fatty acids (VFAs) – have been recognised as viable raw materials for biopolymers

production. Hence, the acidification process that is commonly part of the anaerobic organic waste treatment is capable to provide suitable substrates for PHA bioprocesses. In fact, acidification has been vastly applied in anaerobic treatment of organic residues, although mostly focused on acetic acid production, whilst propionic acid is an undesired compound. However, PHA production in the presence of mixtures of propionate and acetate results in co-polymers with improved commercial features. Operational parameters, namely pH, not only affect the growth of acidogenic microorganisms but also the fermentation products obtained, thus conditioning the VFA yield. Changes observed in the fermentation pathways according to the pH can be attributed to shifts in the dominant population, in the metabolism of the population present or to a combination of both. In addition, temperature, substrate concentration and hydraulic retention time (HRT) influence the pH effect on acidification step, leading to different VFA compositions when different experimental conditions are applied.

The integrated valorisation of organic waste streams, in particular of agro-food by-products, effluents, waste and surplus, with the production of value-added fine chemicals, materials, biofuels and water is a new and challenging development. Organic waste streams are extensively produced in Europe (about 2,500 millions of tons per year) and they are mainly composed of agricultural waste, garden and forestry waste, sludge, food processing waste and organic household waste (about 1,000, 550, 500, 250 and 200 million tons/year, respectively) [2].

Several food companies are currently paying a lot of money for the destruction of their by-products, waste, effluents and surplus. But these are a source of bioactive molecules and biomaterials and, following proper fermentation or bioconversion, of a large array of conventional and new bio-specialties (food ingredients, pharmaceuticals, fine chemicals), biomaterials (biopolymers, lubricants, fibers, pigments, proteins), base chemicals (organic acids, amino acids, vitamins and other metabolites of fermentation) along with biofuels (bioethanol, biogas). Given their biological origin, biodegradability and non toxicity, they are of special interest for the modern food, pharmaceutical, cosmetic, chemical, textile and energy industry. The market of such products is currently increasing enormously worldwide: from 77 to 125 billions € from 2005 to 2010 [2]. Thus, the adoption of such strategies for organic waste valorisation can permit significant improvements in the sustainability and competitiveness of the industrial

sectors mentioned above, by allowing them to better fulfil Europe's vision of a sustainable and competitive knowledge-based economy.

In this study the anaerobic acidification of cheese-whey in a MBBR reactor for further biopolymer production as a waste valorisation methodology was investigated.

2 Bibliographic review

2.1 Acidification process

Anaerobic digestion is an established waste valorisation technology, used for the treatment of a wide variety of organic wastes throughout the decades. It is one of the several biological processing strategies which produce bioenergy and/or bio-chemicals while treating industrial and agricultural wastes. Anaerobic biodegradation can be separated into two phases in order to enhance treatment efficiencies and/or produce bio-products.

Two-phase anaerobic systems have been extensively studied and numerous advantages of phase separation over conventional anaerobic digestion have been described and demonstrated in numerous studies [3]. Some of these advantages include, increased process stability and control, smaller reactor volumes and high tolerance to toxicity and shock loads. These advantages enable the two-phase anaerobic systems to treat many kinds of solid, industrial and agro-industrial wastes such as distillery, landfill leachate, coffee, cheese-whey, dairy, starch, fruit, vegetable solid, food, pulp and paper, olive mill, abattoir, dye wastewaters, primary and activated sludge [3].

The anaerobic acidification could be useful for the production of organic acids (e.g. VFAs), which have variety of industrial uses [3].

2.2 Moving bed bioreactor

The MBBR concept was conceived in Norway during the 1980's, in response to agreements by eight European nations to reduce nitrogen loadings to the North Sea. Towards this end, the Norwegians focused on developing compact, small footprint, low maintenance attached growth systems that minimized the operational and maintenance issues associated with trickling filters and rotating biological contactors. The first MBBR facility became operational in 1990 in Lardnal, Norway. MBBR technology has since made significant penetration into the European market with an installed base of more than 300 MBBR systems [4].

MBBR systems are based on reactors that are filled with plastic carriers to provide a

surface that is colonized by bacteria that grow into a biofilm [4].

The carrier elements provide a large protected surface area for biofilm growth and optimal conditions for the bacteria culture to grow and thrive. The biofilm that is formed around each carrier element protects the bacterial cultures from being washed out of the system. The biofilm also provides a more stable “home” for bacteria growth, so there is less space required when compared to other biological systems and far less controls [5].

The MBBR reactors can be operated under aerobic conditions for BOD removal and nitrification or under anoxic conditions for denitrification. During operation, the carriers are kept in constant circulation. In an aerobic reactor, circulation is induced through the action of air bubbles injected into the tank by a coarse bubble diffuser system. In an anoxic reactor, a submerged mixer is typically supplied. The carriers can occupy up to 70% of the reactor volume on a bulk volume basis. Experience has shown that mixing efficiency decreases at higher percentage fills [4].

Because MBBR is primarily an attached growth process, treatment capacity is a function of the specific surface area (SSA) of reactor. The SSA for a reactor is calculated as the quotient of the total surface area of the carrier that is available for biofilm establishment and the reactor volume. The media SSA reflects the amount of surface area available for biofilm development per unit volume of the media, on a bulk volume basis. The reactor SSA equals the SSA of the media multiplied by the fraction of the total reactor volume that the media occupies (bulk volume basis). The MBBR has a greater performance potential than conventional fixed film type processes [4].

2.3 Production of polyhydroxyalkanoates (PHAs)

Polyhydroxyalkonoates (PHAs) are a group of polyesters that is a promising alternative to conventional plastics due to their biodegradability and capability of being produced from renewable resources. PHAs are an example of bioplastics which consists of polyesters of various hydroxyalkonoates and synthesised by a wide variety of microorganisms.

In recent years, there has been a great interest in investigating potential alternative processes for PHA production aiming at decreasing the biopolymer production costs. Those strategies include the use of low valued substrates as waste feedstocks and microbial mixed cultures [6]. Selection of a suitable substrate is an important factor for PHA optimisation, inducing their content, composition and polymer properties [7].

PHA synthesis from carbohydrate-rich substrates requires a previous anaerobic fermentation step to transform the sugar content into FVAs [8]. In this sense, anaerobic fermentation can be applied as a pre-treatment process to convert various organic compounds into VFAs, which will increase the potential to produce PHA from organic wastewaters. As the monomer composition affects the physical and mechanical properties of PHAs, the composition of the VFAs produced during fermentation will influence the final polymer product.

3 Objective of the study

The objective of this research is to study the acidogenic fermentation of cheese-whey in order to optimise the operational conditions from the point of view of its potential to produce VFAs as substrates for PHAs production.

The experiments will be performed in order to provide information on the amount of the organic matter that can be converted to volatile organic acids, which are the main substrates for polymer production and also to identify which acids predominate in the acidogenic effluent.

Referring to the specific objectives the answers should be given to the following questions:

1) how to maximise VFA production (mainly, of Acetic, Propionic and i-Butyric acids) and identify the types of acids;

2) how to decrease methane production.

4 Methodology of the study

4.1 The substrate characterisation

There are different types of organic sources that can be converted to VFAs. Cheese-whey is a by-product from dairy production, which is produced in enormous quantities resulting from cheese and casein manufacture. Cheese-whey is the serum part of milk remaining after separation of the curds during casein or cheese making. It is rich in nutrients and contains at least half the solids found in whole milk – Table 4.1. In addition, cheese-whey has a very strong polluting capacity, with a biological oxygen demand (BOD) from 40,000 to 45,000 mg/L. On a worldwide scale, only 50% (v/v) of total cheese-whey produced is utilized, and the remaining is either discharged into the sewerage or ocean outfall, or sprayed on pastures [9].

Tab. 4.1 – Composition of the cheese-whey broth medium [9]

Cheese-whey constituents	Amount (%, w/v)	Standard deviation
Lactose	4.91	0.3
Proteins	0.93	0.05
N_2	0.13	0.04
Fats	0.71	0.01
Ca	4.95 ppm	0.1
K	0.93 ppm	0.04
Na	0.078 ppm	0.07
Total solids	6.25	0.05

As it can be seen in Table 4.1 cheese-whey is composed of lactose, proteins and fat that may be used by microorganisms as carbon and nitrogen sources for metabolism to support growth and biopolymer production. Lactose is the most abundant constituent of cheese-whey and it forms at least 78% (w/w) of the cheese-whey's total solids [9], which is readily available substrate for anaerobic bacteria.

The main characteristics of cheese-whey used in the experiments are described in Table 4.2.

Tab. 4.2 – The main characteristics of cheese-whey used in the experiments

Parameter	The characteristics of cheese-whey as	
	dry powder	100 g of cheese-whey solution
tCOD, gO_2/g	1.075	-
pH	-	6.28
alkalinity, $gCaCO_3/L$	-	2.22
HAc, g/L	-	2.29
HPr, g/L	-	0.40

More experience in the production of PHAs by activated sludge microorganisms from cheese-whey is needed in order to establish the practical potential of the concept and to identify aspects of the process that are important with respect to process stability, control and optimisation from perspectives of cheese-whey disposal and polymer production.

Hence, cheese-whey was used in continuous fermentation experiments where the influence of several parameters such as HRT, alkalinity and pH on VFA production yield and VFA composition were examined.

To find out the parameters, which influence most the process of acidic fermentation and determine VFA conversion a mass balance of the system (4 steps) was also performed.

The parameters to be studied are: alkalinity and pH by controlling alkalinity addition; HRT by controlling the volumetric load in terms of flowrate; and OLR by controlling substrate concentration in the feed to the reactor. According to the parameters to be controlled there were two sets of experimental conditions applied: one set with various OLRs and another with different alkalinity values for a chosen OLR.

The first set of experimental conditions was mainly performed in order to evaluate the influence of the applied OLRs on the behaviour of the MBBR system. It consisted of 4 phases, each one with a different OLR at a constant value for HRT, which was equal to 12 h.

This study aims to increase the acidification degree as much as possible [10], at a constant alkalinity of 3.6 $gCaCO_3/L$ (3.0 $gNaHCO_3/L*d$).

Phase 1: OLR = 20 gCOD/L*d;

Phase 2: OLR = 30 gCOD/L*d;

Phase 3: OLR = 60 gCOD/L*d;

Phase 4: OLR = 35 gCOD/L*d.

The secondary set of experiments studied was concerning different inlet alkalinity at a stable OLR equal to 30 gCOD/L*d.

Phase A: alkalinity = 3.6 $gCaCO_3/L*d$ (3.0 $gNaHCO_3/L*d$);

Phase B: alkalinity = 4.1 $gCaCO_3/L*d$ (3.5 $gNaHCO_3/L*d$).

During the research it was also studied another criterium concerning hydraulic parameters such as the mixing system in the MBBR reactor.

4.2 The experimental set-up

The scheme of experimental set-up is presented below on the Figure 4.1.

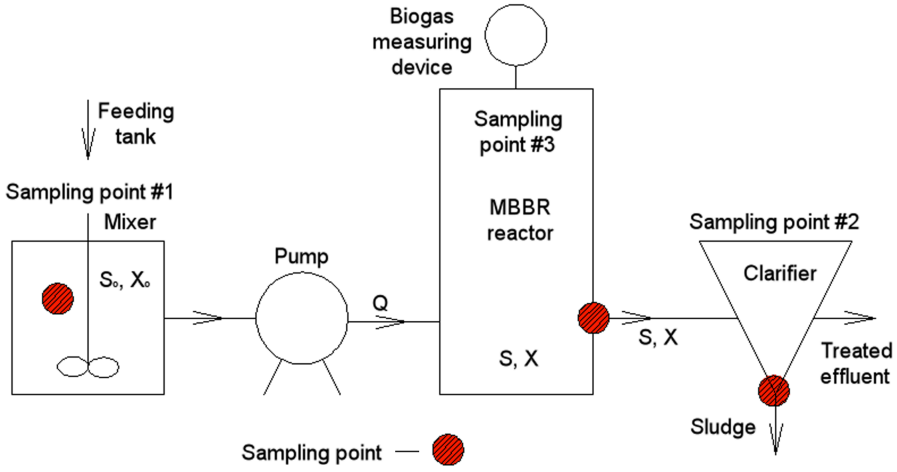


Fig. 4.1 – The scheme of experimental set-up and location of sampling points

The experimental set-up consists of the following units:

1) a tank with a mixer where the feeding for bacteria was prepared;
2) a pump which transfers the feeding to the bioreactor;
3) the MBBR reactor with a mixer and a biogas measuring device;
4) a clarifier.

Figure 4.2 shows a picture of the real conditions of the set-up.

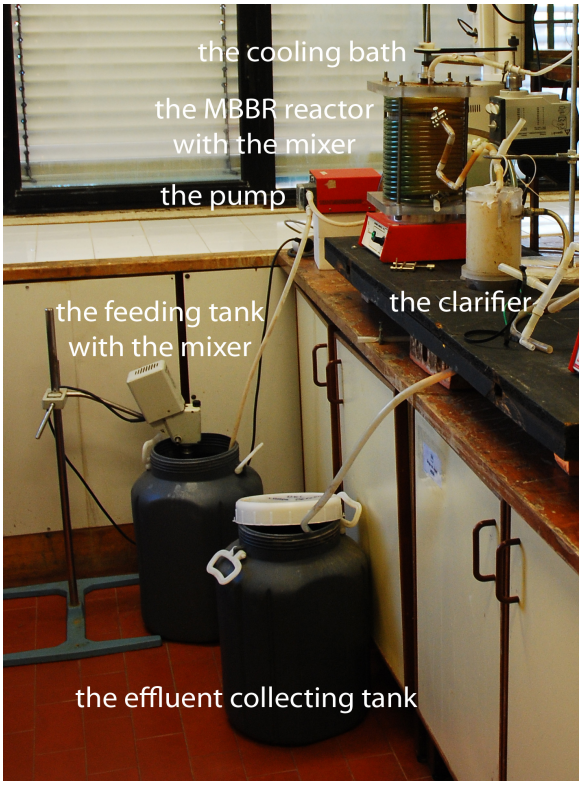


Fig. 4.2 – The set-up in the real conditions

The carrier element used for this experiment is called “Biobilm 9”, and it can be seen in Figure 4.3.

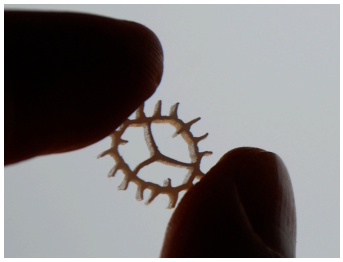


Fig. 4.3 – A view of carrier “Biofilm 9”

The feeding solution was mixed at the 9th speed of a mixer Heidolph RZR 2020 at the range 1 which resulted in 330 rpm permanently. The mixing process inside the reactor was set at the 5th speed at the range 1 of the same brand of mixer – Heidolph RZR 2020 resulting in 130 rpm constantly.

Acidification tests will be performed in the MBBR reactor fed with the previously selected substrate (cheese-whey), at mesophilic temperature. The operational parameters studied include: pH by changing alkalinity; HRT by controlling the flowrate and organic loading rate (OLR) by controlling substrate concentration.

The operational conditions of the set-up to be studied are described in Table 4.3.

Tab. 4.3 – The operational parameters and phases of the experiments

Phases (first set of experiments)	1	2		3	4
Phases (secondary set of experiments)	-	A	B	-	-
V reactor (L)	2.54				
OLR (gCOD/L*d)	20	30		60	35
HRT (d)	0.5				
Q (L/d)	5.0				
Alkalinity ($gCaCO_3$/L)/ ($gNaHCO_3$/L)	3.6 /3.0	3.6/3.0	4.1/3.5	3.6/3.0	3.6/3.0
V clarifier (L)	1.0				

The feed is composed of cheese-whey, sodium bicarbonate, macro- and micronutrients and inorganic salts.

In Table 4.4 are described the amounts of compounds and nutrient solutions added to the water taken from the tap to prepare the feeding solutions.

Tab. 4.4 – The composition of MBBR feed

FEED components	Feeding for 2 days				
	OLR = 20 gCOD/L*d; alk_{in} = 3.6 $gCaCO_3$/L	OLR = 30 gCOD/L*d; alk_{in} = 3.6 $gCaCO_3$/L	OLR = 30 gCOD/L*d; alk_{in} = 4.1 $gCaCO_3$/L	OLR = 60 gCOD/L*d; alk_{in} = 3.6 $gCaCO_3$/L	OLR = 35 gCOD/L*d; alk_{in} = 3.6 $gCaCO_3$/L
V water (L)	10				
m whey (g)	99	148	148	297	173
m $NaHCO_3$ (g)	31	31	36	31	31
Micro-nutrients (mL	10				
Macro-nutrients I (mL)	10				
Macro-nutrients II (mL)	10				

1 mL of solutions of micro- and macronutrients I, II for anaerobic processes was accounted for 1 L of feed.

The micro- and macronutrients' constituents are given in the next table – Table 4.5.

Tab. 4.5 – The content of micro- and macronutrients solutions

The solution content of		
micronutrients, mg/L	macronutrients I, mg/L	macronutrients II, mg/L
$FeCl_3.6H_2O$ – 2454	NH_4Cl – 170	$CaCl_2.2H_2O$ – 8
$CoCl_2.6H_2O$ – 2000	KH_2PO_4 – 37	$MgSO_4.4H_2O$ – 9
$MnCl_2.4H_2O$ – 500		
$CuCl_2.4H_2O$ – 30		
$ZnCl_2$ – 50		
H_3BO_3 – 50		
$(NH_4)_6Mo_7O_{24}.4H_2O$ – 90		
EDTA – 1000		
$NiCl_2.6H_2O$ – 50		

To evaluate the acidification potential of cheese-whey it was necessary to perform the following analyses:

– COD tests to analyse the organic substrate: samples for the total COD (tCOD) were taken in the feeding tank to assess the total amount of organic matter present; samples for the soluble (sCOD) were taken in the treated effluent from the clarifier to know the amount of remaining soluble organic matter and compare the values with the amounts of VFAs produced during the acidification process. For that goal the VFA analysis was also conducted for the effluent treated.

– TSS and VSS analyses were performed in order to characterise the biomass, evaluate its growth rate and identify the ratio of VSS to TSS. The tests were done for a sample taken from the MBBR reactor and clarifier after mixing the whole content.

5 Experimental methods

The experiment was started on the 17nd of January, 2011, and finished on 3th of June, 2011. Sampling was performed twice per week – mainly, on Mondays and Thursdays (with time periods of 3 and 4 days, respectively). The whole period of studying and evaluating the bioreactor performance for the treatment of cheese-whey with respect to its acidic fermentation ability took around 137 days. It resulted in 39 sampling days (I1-I39).

As it was already mentioned there were conducted the following analyses: tCOD, sCOD, TSS, VSS, VFA, pH and alkalinity.

After obtaining the data from all analyses and measurements, it was applied empirical formulas which converted methane, biomass and VFA productions into COD units in order to close a mass balance of the system and obtain the efficiency of the biological system for all conditions applied during the whole period of the experiment (Phases 1-4 and Phases A-B).

The procedures for the analyses to be done are described below if not referred to the literature source.

5.1 COD test

Two types of COD tests were performed – the tCOD test for a sample taken from the feeding tank ($tCOD_{in}$) and the sCOD for a sample taken from the treated effluent discharged from the clarifier ($sCOD_{out}$).

For the COD tests the «closed reflux colorimetric method» was used [11].

The only difference in the procedures described in the literature was the necessity to dilute samples for both tCOD and sCOD tests for feeding and treated effluent, respectively, because these values were higher than the range that could be detected by the spectrophotometer AQUALYTIC® (PC compact, COD vario).

Hence, samples taken from the feeding tank, clarifier and MBBR reactor (in the range of 1.5-0.5 mL) were diluted with distilled water until 50 mL. The dilution factor was equal to 1:33 and 1:100, respectively. Each COD test was replicated three times (the data obtained can be found in Appendix #2.1).

5.2 TSS and VSS analyses

The TSS analysis was done according to the standard methods [12] and performed in triplicate (Appendix #4.1-4.3 and #5.1-5.3). The calculations were computed in the following way:

mg TSS/L = ((A-B)*1000)/sample volume, mL,

where:
A – weight of (a dish with a filter + dried residue), mg;
B – weight of a dish with a filter, mg.

The other parameters were calculated according to the formulas from [12]:

mg VSS/L = ((A'-B')*1000)/sample volume, mL;
mg FSS/L = ((B'-C')*1000)/sample volume, mL,

where:
A' – weight of (a dish with a filter + dried residue) before ignition, mg;
B' – weight of (a dish with a filter + dried residue) after ignition, mg;
C' – weight of a dish with a filter, mg.

5.3 pH and alkalinity tests

pH of a sample was measured with the pH-meter «Consort 5100».

The titration of a sample for alkalinity analysis was done with H_2SO_4 with normality 0.1mol/L until the pH dropped down to 4.50. The alkalinity of a sample was measured in mg of $CaCO_3$/L and the formula that was used for the calculations is [13]:

Alkalinity, mg $CaCO_3$/L = (A*N*50000)/mL sample,

where:
A – the amount of sulfuric acid used, mL;
N – normality of sulfuric acid solution, mol/L.

The data on pH, inlet and outlet alkalinity can be found in Appendix #2.1.

5.4 VFA test

To detect the VFAs gas chromatograph CHROMPACK CP9001 with FID detector was used.

For this analysis a sample of 5 ml of filtrated effluent from MBBR was taken. Then 0.5 ml of formic acid was added and put into the gas chromatograph to detect the types of volatile fatty acids produced in the reactor and their concentrations (Appendix #1.1 and #1.2).

5.5 Mass balance analysis

To visualise the mass balances of the system it was necessary to build a scheme for the organic matter degradation. There is a general picture of organic degradation – in Figure 5.1.

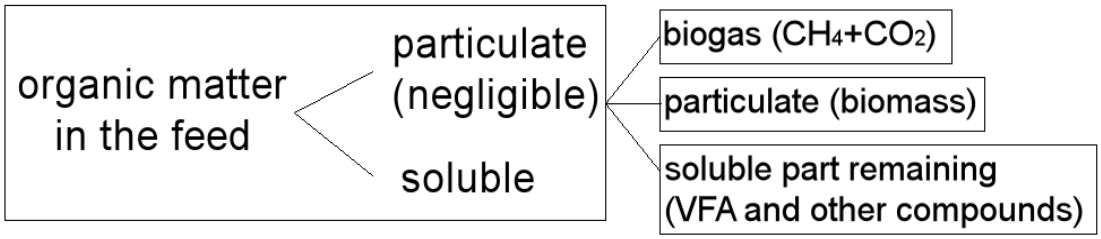


Fig. 5.1 – The scheme of organic matter degradation

The organic matter, which is presented as COD in the feed is being converted into different structural units as biogas (CH_4 and CO_2), biomass and soluble organic matter

during the process of treating cheese-whey in an acidogenic MBBR reactor.

Hence, first it was necessary to convert all constituents into COD units using the next calculations:

1. the amount of methane produced and measured as theoretical COD (thCOD) can be calculated through the following stoichiometric equation:

$CH_4 + 2O_2 - > CO_2 + 2H_2O$

This relation is determined by balancing the simplified typical structure of CH_4 as follows:

$COD = m_{O2} / m_{CH4} = 64\ g / 16\ g = 4\ g\ O_2 / g\ CH_4$.

As 1 mole of gas = 22.4 dm^3 at standard temperature and pressure (STP), hence, at T = 308 K, 1 mole of gas = 25.3 dm^3.

And 1 g of CH_4 occupies $V_{CH4} = 25.3 / 16g = 1.6\ dm^3\ CH_4$.

To summarise, 1 g of CH_4 is 4 g of COD and occupies 1.6 dm^3 CH_4 at T = 308 K, therefore, per 1 g of COD: Y= 1.6 dm^3 CH_4 / 4g COD = 0.4 m^3 CH_4 / kg COD. Hence, the formula for calculation of methane amount as COD per day is the following:

$$m_{CH4}\ [gCOD/d] = 2.5\ [g/L] * V\ [L/d] \quad (5.1)$$

The calculated amounts are presented in Appendix #3.1.

2. the same approach was used to convert the biomass into thCOD:

$C_5H_7NO_2 + 5O_2 - > 5CO_2 + 2H_2O + NH_3$,

where $C_5H_7NO_2$ represents the biomass:

$COD = m_{O2} / m_{C5H7NO2} = 160\ g / 113\ g = 1.42\ g\ O_2 / g\ C_5H_7NO_2$.

Therefore, when the biomass is oxidised, it takes 1.42 g O_2 per 1 g VSS. In other words, the formula looks like this:

$$m_{bio}\ [gCOD/L] = 1.42\ [g/g] * m_{VSS}\ [g/L] \quad (5.2)$$

The calculations are in Appendix #4.1-4.3, 5.1-5.3.

3. in order to calculate the amount of VFAs and convert them into COD, it is

necessary to follow the next steps:

- first, to get the chromatograph calibration curves;
- then, to obtain the coefficients “m” and “b” (can be seen in Table 5.1 and Figure 5.2) from that calibration curves and transform data from chromatograms into VFAs concentration, mg/L, using the following equation:

  $y = mx + b$,

where:

y – area, obtained from the chromatograms, μV*min;

x – VFA concentration, mg/L.

- afterwards, to convert the VFAs concentration into its concentration as COD, mg COD/L, using the calculations of theoretical oxygen demand (thOD) of each VFA detected – in Table 5.2.

From the calibration curves the coefficients «m», «b» obtained are described in Table 5.1.

Tab. 5.1 – The values of coefficients «m», «b» and other parameters and limits of VFA chromatograms

VFA	**m**	**b**	Retention time (average ± standard deviation) (min)	Detection limit (mg/L)	Quantification limit (mg/L)	r^2
Acetic	**452.72**	**80059**	3.142 ± 0.135	24	72	0.986
Propionic	**925.99**	**59328**	4.027 ± 0.200	27	81	0.998
i-Butyric	**1054.2**	**105437**	4.982 ± 0.176	26	78	0.995
n-Butyric	**1121.2**	**79591**	5.428 ± 0.181	21	63	0.995
i-Valeric	**1337.4**	**185078**	6.475 ± 0.201	38	114	0.992
n-Valeric	**1262.3**	**129556**	7.200 ± 0.193	43	129	0.972
n-Caproic	**1241.1**	**1052**	9.073 ± 0.313	85	255	0.996

The types of acids were identified due to their correspondence to those peak areas according to the retention time of each one.

A typical chromatogram obtained for the 11th day of sampling is shown in Figure 5.2.

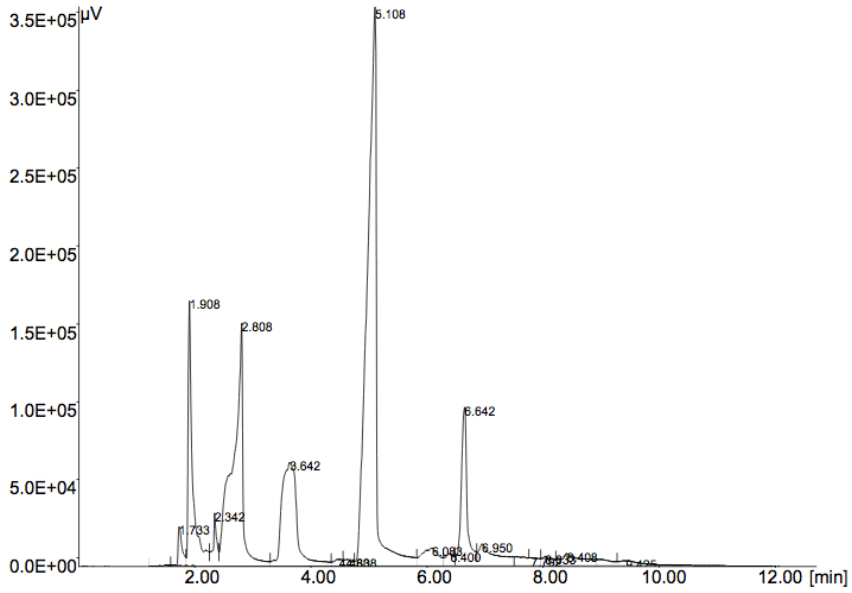


Fig. 5.2 – The chromatograph of the 11th day of sampling

After the chromatograms were gotten it was needed to solve the equation y = mx + b to find out the concentration of each VFA produced:

area [µV*min] = m * VFA [mg/L] + b =>

VFA [mg/L] = (area [µV*min] - b)/m

Finally, it was necessary to convert VFA, mg/L, into COD, mg COD/L, in the following way:

$$\text{VFA [mgCOD/L]} = \text{VFA [mg/L]} / \text{thOD}_{acid} \quad (5.3)$$

For that purpose the thOD was calculated according to the stoichiometric equations for each acid as it is shown on an example for the Acetic acid:

$CH_3COOH + 2O_2 \rightarrow 2CO_2 + 2H_2O$

$thOD = (Mw_{O2} * n_{O2}) / Mw_{CH3COOH} = (32 * 2) / 60 = 1.067$ g

Therefore, the results for all calculations are described in Table 5.2.

Tab. 5.2 – The thOD calculated for each VFA

thOD of VFAs, g						
Acetic	Propionic	i-Butyric	n-Butyric	i-Valeric	n-Valeric	n-Caproic
1.067	1.514	1.818	1.818	2.039	2.039	2.207

The calculations of VFAs amounts as COD are also given in Appendix #1.1 and #1.2 in tables.

6 Results and discussion

The results of the experiments are described in the following graphs and tables.

As it was already mentioned the experiment consisted of 4 phases. In Table 6.1 it can be seen the whole of experimental period and real conditions applied.

Tab. 6.1 – The schedule of experimental frameworks

Phase of experiment	Phase 1	Phase 2A	Phase 2B	Phase 3	Phase 4
Duration of experiments, days	36 (17.01.2011 - 22.02.2011)	28 (22.02.2011 - 22.03.2011)	10 (22.03.201 - 01.04.2011)	30 (01.04.2011 - 01.05.2011)	34 (01.05.2011 - 03.06.2011)
Operational parameters	OLR = 20 gCOD/L; alk_{in} = 3.6 $gCaCO_3$/L	OLR = 30 gCOD/L; alk_{in} = 3.6 $gCaCO_3$/L	OLR = 30 gCOD/L; alk_{in} = 4.1 $gCaCO_3$/L	OLR = 60 gCOD/L; alk_{in} = 3.6 $gCaCO_3$/L	OLR = 35 gCOD/L; alk_{in} = 3.6 $gCaCO_3$/L

It is important to notice that during Phase 2 the mixer in the MBBR reactor started malfunctioning. In the end of Phase 3 the reactor was cleaned and the mixer in the MBBR was fixed up.

The value of OLR was chosen on basis on the data obtained during previous phases of experiment which resulted in applying OLR of 35 gCOD/L*d and alkalinity of 3.6 $gCaCO_3$/L during Phase 4. Hence, the final experimental phase – Phase 4 – was conducted in order to evaluate the MBBR performance and, especially, the effect of content mixing on the reactor performance.

In order to provide the feed of the MBBR reactor with different OLRs at a constant HRT the flowrate has to be determined. The flowrate needed for the experiment was 5.0 L per day. So, the feeding pump has to be calibrated with fresh water in order to obtain the pump calibration curve. After starting the experiment, it was also necessary to adjust the feeding pump flowrate with ready prepared cheese-whey solution. Nevertheless, it

figured out that the average flowrate during the experiment was lower and about 3.55 L per day. This value was then used in all calculations.

Hence, it was necessary to recalculate the OLRs applied to the reactor due to the fact that the real flowrate was equal to 3.55 L/d. The corrected OLRs are presented in Table 6.2.

Tab. 6.2 – The real OLRs according to the experimental data obtained

	OLR, gCOD/L*d			
Value expected	20	30	60	35
Value real	**22**	**33**	**66**	**35**

To evaluate the MBBR performance regarding cheese-whey acidification the results obtained from all conducted tests are described and analysed according to the specific objectives of the study.

6.1 pH, alkalinity and VFA evolution

Data on alkalinity of inlet cheese-whey solution (alk_{in}) and pH and alkalinity of outlet (alk_{out}) and VFA in the reactor are described in Figures 6.2 and 6.3.

Figure 6.2 represents the whole continuous period of the experiment with all 4 phases.

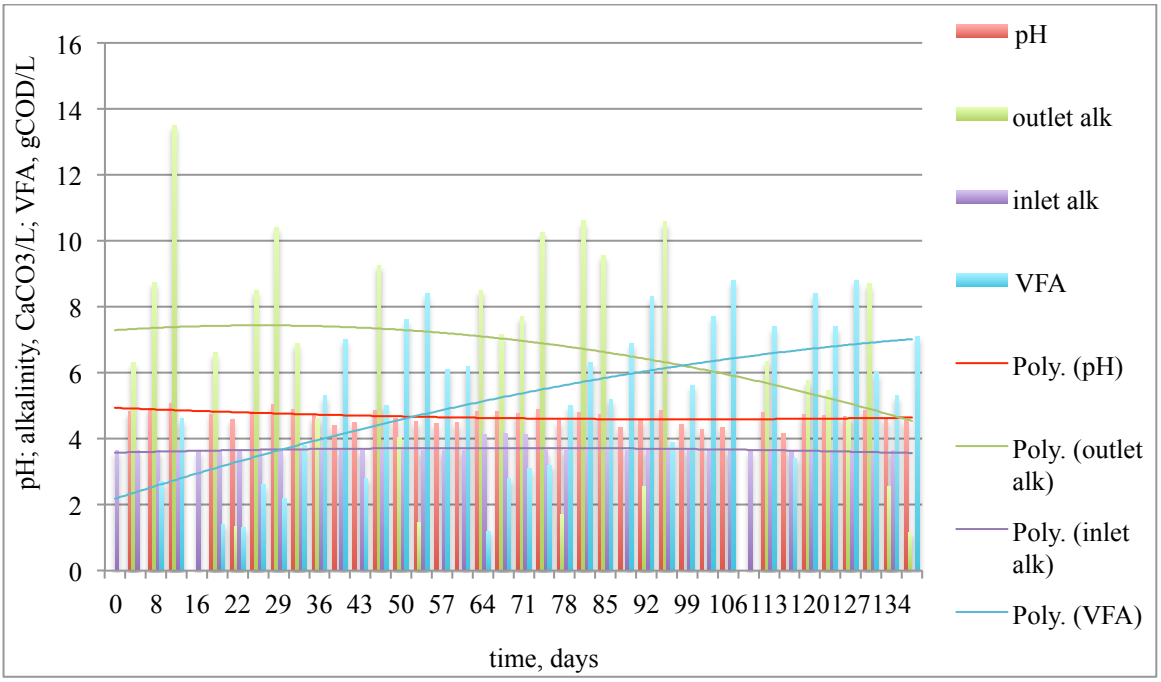


Fig. 6.1 – average values of pH, alk_{out}, alk_{in} & VFA data vs. time during the whole of experiment

It should be pointed out that according to Figure 6.1 the pH varies from 4.17 to 5.08; the mean value of inlet alkalinity is 3.67 $gCaCO_3/L$ and it varies from 3.63 to 4.17 $gCaCO_3/L$; the mean value of outlet alkalinity is 6.9 $gCaCO_3/L$ and it varies from 1.15 to 13.5 $gCaCO_3/L$; the VFA average is 4.7 gCOD/L and it changes from 1.2 to 8.8 gCOD/L.

This situation can more clearly be seen considering Figure 6.3 with the parameters depiction for each phase separately.

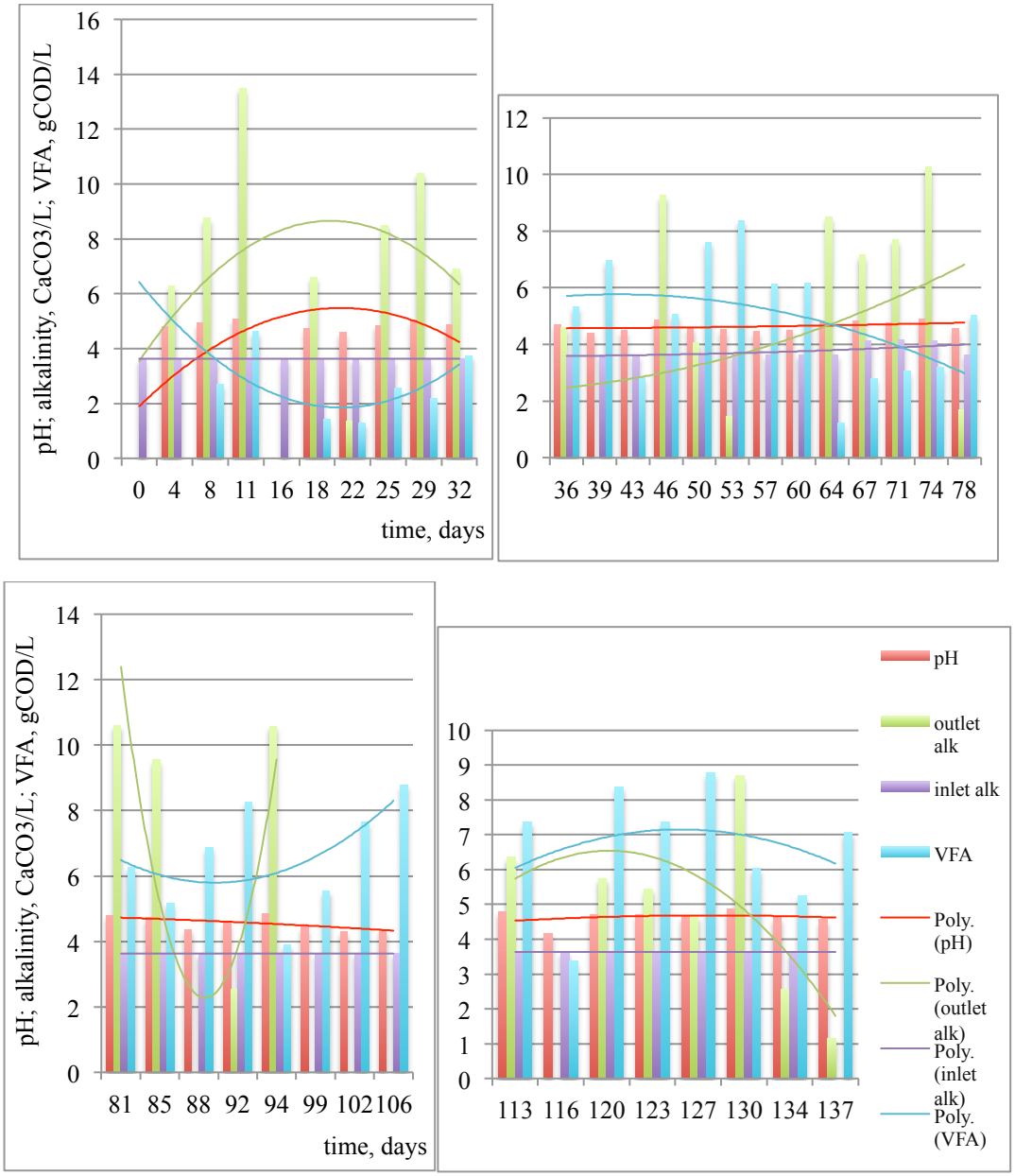


Fig. 6.2 – pH, alk_{out}, alk_{in} & VFA data vs. time during the 1st (left, up) and 2nd (right, up) the 3rd (left, down) and 4th (right, down) phases of experimental time

The parameter average values of alkalinity in the feed and VFA and alkalinity in the reactor are given in Table 6.3.

Tab. 6.3 – Phases and parameters of experiment

Parameter	Phases of experiment			
	Phase 1	Phase 2	Phase 3	Phase 4
pH	4.59-5.08	4.40-4.90	4.29-4.85	4.17-4.86
average alk_{out}, $gCaCO_3/L$	7.4	6.9	7.0	4.9
average alk_{in}, $gCaCO_3/L$	3.6	3.8	3.6	3.6
average VFA, gCOD/L	2.7	4.9	6.6	6.7

According to the 1st picture of Figure 6.2, which represents Phase 1 (left, up) it is possible to conclude that pH is kept at relatively stable low values (4.6-5.1). Although the inlet alkalinity is maintained constantly, the outlet alkalinity is not stable, presenting higher values due to the low VFA rate. The outlet alkalinity presents a cyclic character. It decreases in amount proportionally to the VFA increase. It is important also to mention that the amount of VFAs does not change much and keeps staying quite low with an average value of 2.7 gCOD/L.

The 2nd picture (right, up) presents the amount of VFAs produced during Phase 2. In comparison with the 1st picture pH is higher and the VFAs' level is also higher with an average value of 4.9 gCOD/L. At the same time at increasing the VAFs the outlet alkalinity decreases (from 7.4 to 6.9 $gCaCO_3/L$).

During Phase 3 (left, down) there is an increase of VFA production comparing to Phase 2 with an average of 6.6 gCOD/L which seems to be also cyclic. Here it can also be seen that the outlet alkalinity does not decrease with the VFA increase, moreover, it slightly increases. Probably, the outlet alkalinity at this phase (Phase 3) was influenced by increased value of inlet alkalinity at the end of Phase 2. pH shows a decreasing tendency during the whole period of the phase.

Phase 4 (right, down) presents data with the highest amount of FVAs (6.7 gCOD/L) with the lowest outlet alkalinity (4.9 $gCaCO_3/L$). The outlet alkalinity behaves depending on the amount of VFAs present during almost the whole period of this phase.

The pH inside the MBBR reactor was always acidic and changed from 4.2 to 5.1 during all the experimental period.

6.2 Organic matter degradation

One of the most important analyses to understand the organic matter degradation was the COD analysis. The COD analysis characterises the substrate in the feed which is mainly composed of cheese-whey and $NaHCO_3$. The tCOD provides with information about the amount of oxygen which is needed to oxidise both particulate and soluble organic compounds in a sample, whereas the sCOD reveals the amount of oxygen which is needed to oxidise the soluble organic matter in a sample.

Hence, the $tCOD_{in}$ was performed to determine the amount of organic matter in the feed, which could be oxidised to the final products. The $sCOD_{out}$ was done to find out the amount of organic matter, which was converted to the final products which also allows a comparison with the amount of VFAs produced.

Finally, it was possible to evaluate the COD removal rate. The data are presented for each phase of the experiment in Table 6.4.

Tab. 6.4 – The COD removal rate at different periods of experiment

Phases	COD removal average, %
Phase 1	22
Phase 2	24
Phase 3	22
Phase 4	20

According to Table 6.4 it is seen that the highest level of treatment was reached during Phase 2 – on average 24% of COD removal. It is important to mention that the COD removal parameter shows the global efficiency of the MBBR reactor.

The FVAs production efficiency can be evaluated according to the ratio of VFAs produced to COD_{in} or COD_{out} data – Figure 6.4.

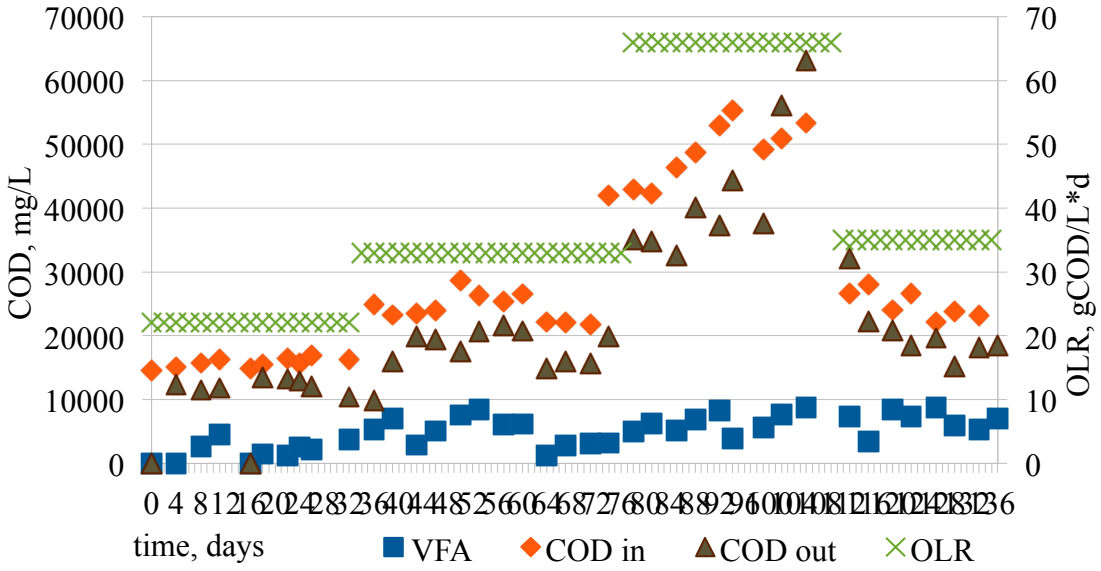


Fig. 6.3 – The correlation of VFAs amount, COD_{in} and COD_{out} data vs. time

According to Figure 6.3 it can be seen that the amount of FVAs produced are not very high, lower than 10000 mg/L as COD during the whole period of experiment. The most efficient period of the MBBR reactor performance seems to be the last one – Phase 4 for an OLR = 35 gCOD/L*d. COD_{in} and COD_{out} values varies not a lot and are quite similar in between (Phases 2A and 4). Although this, values of COD_{in} and COD_{out} of Phase 4 mostly are smaller than the ones of Phase 2A. Given that the FVAs are almost the same in its amounts during both phases, so, the ratios between VFAs and COD parameters are higher for Phase 4 which corresponds to a better result of the MBBR reactor performance.

To assess the effectiveness of the set-up for the acidification of cheese-whey more properly Figure 6.4 was depicted. It presents the ratios between VFAs production and COD_{out} and COD_{in} and ratio of COD_{out} to COD_{in} in order to see the effective yield in VFAs production within the full time of experiment.

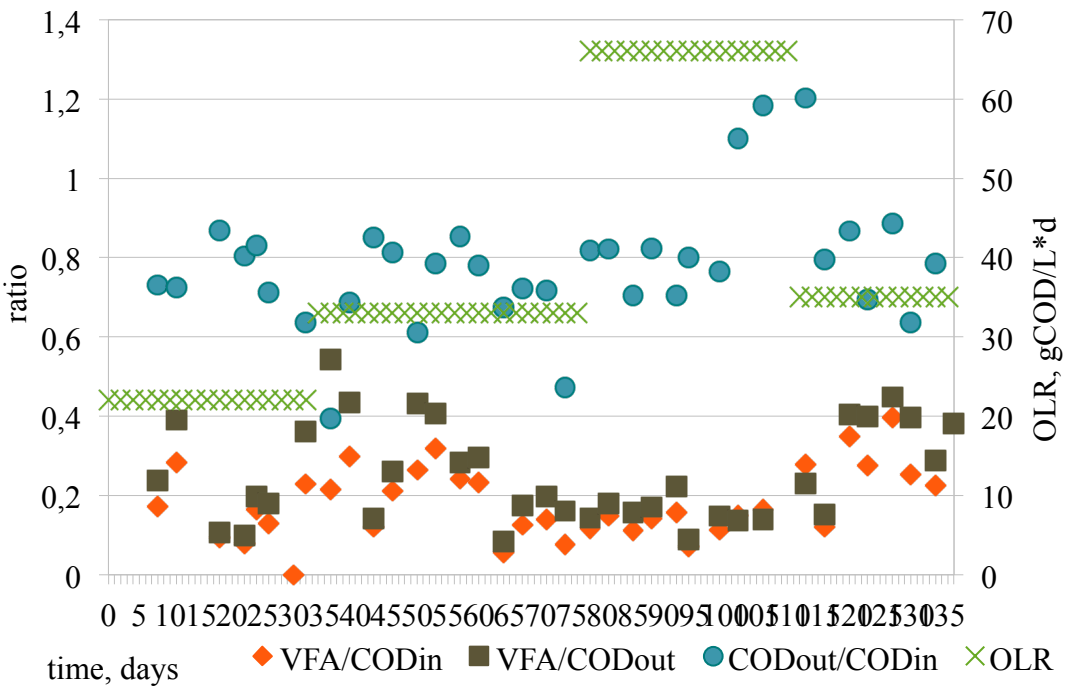


Fig. 6.4 – The relation between VFAs and COD_{in}, VFAs and COD_{out}, COD_{out} and COD_{in} vs. time

In Figure 6.4 it can be easily seen that the highest ratios of FVAs towards COD_{out} and COD_{in} are during Phases 2 at OLR = 33 gCOD/L*d and 4 at OLR = 35 gCOD/L*d.

In both Figures 6.3 and 6.4 it can be seen that in the end of Phase 3 COD_{out} is higher than COD_{in} ($COD_{out}/COD_{in} > 1$). Apparently, this happened because at Phase 3 the OLR was very high (66 gCOD/L*d) which made the MBBR reactor to be overloaded: some of the organics present in the feed started to accumulate in the reactor which was bad to the retention of high amount of suspended solids as it could be seen in the TSS analyses during that time. Afterwards, it was necessary to open the reactor and clean it.

The amount of individual volatile fatty acids expressed as COD during all experiments are presented according to their amounts in mgCOD/L – Figure 6.5.

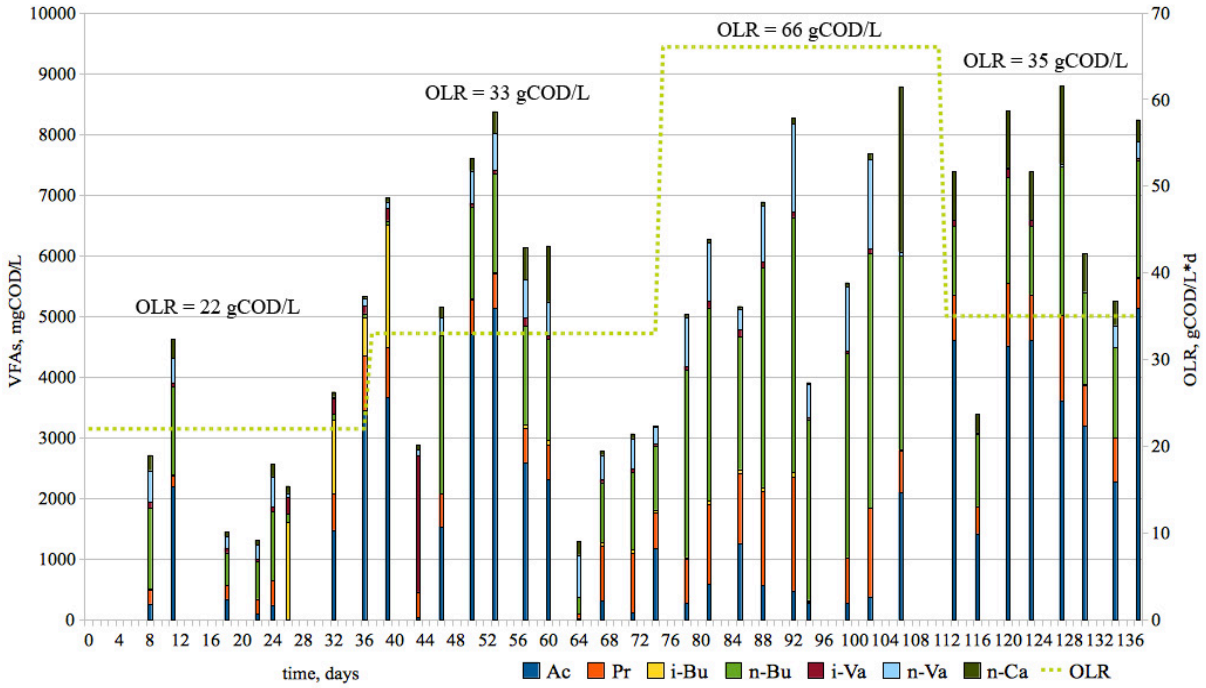


Fig. 6.5 – The distribution of VFAs, mgCOD/L

Figure 6.6 shows the contribution of each acid to its total amount as percentage.

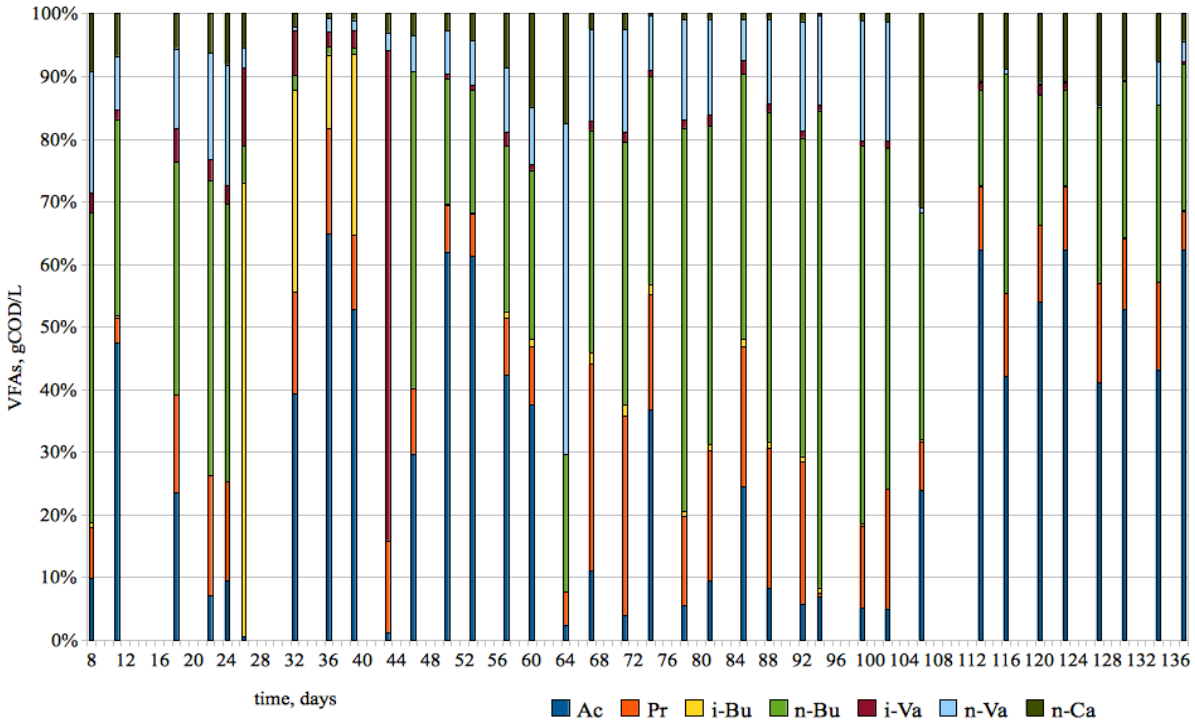


Fig. 6.6 – The distribution of VFAs, %

According to Figures 6.5 and 6.6 it is possible to study the effect of the operational

conditions, especially, organic loading rate on the VFA yield.

Both figures present the VFAs distribution during all 4 phases of experiment. It can be seen that the amounts of VFAs during Phase 1 are the lowest ones. The amounts of each acid vary a lot during that period. The reactor just started working at the load of 22 gCOD/L*d, so, the steady state was not yet reached. However, in the end of this phase the i-Butyric acid (one of concern to the specific objectives of this study) was predominant.

Higher amounts of VFAs are observed in the next period of experiment (Phase 2). In this phase the predominant acids are Acetic and n-Butyric. It is important to notice that in the beginning of this phase there is still comparatively high amount of i-Butyric acid. Apparently, an increase in the OLR affected its production in a bad way.

During the 3rd phase VFAs production either maintained the same level or increased slightly. However, the individual VFAs content is very different, where n-Butyric acid dominates completely over the others. The highest amounts of individual VFAs produced during this phase are n-Butiric, n-Valeric and Propionic acids. The Acetic acid is presented but always in small amounts.

The 4th phase can be characterised as a phase with the highest amounts of Acetic acid (about 50% of all VFAs content). There is still a significant amount of n-Butyric acid and smaller amount of Propionic one.

The i-Butyric acid in high amounts was observed just only in the end of 1st phase and in the very beginning of the 2nd one. During other phases it either does not appear at all or appears in very insignificant amounts. i-Valeric and n-Caproic acids appear constantly but always in negligible amounts.

The produced VFAs may be converted to PHAs by PHA accumulating bacteria. In this case, the monomer composition of the produced PHAs depends on the types of VFAs that are being consumed. Acetate and butyrate have a tendency to form hydroxybutyrate (HB) monomers whereas the presence of propionate tends to increase the amount of hydroxyvalerate (HV) in the polymer. In turn, the physical and mechanical properties of

PHAs are dependent on the monomer composition of the polymer. So, here is an interest to get specific VFAs in order to obtain polymers with better quality characteristics – pure polyhydroxybutyrate (PHB), a common form of PHA, is stiff and brittle but with the incorporation of HV, a copolymer (PHB-co-HV) is formed that is more elastic and flexible [14].

Hence, according to the VFAs distribution obtained in the experiment and regarding the influence of monomer composition of the polymers on physical and mechanical properties of PHAs, it is possible to conclude the following: the better VFAs content is presented during Phase 4 at an OLR = 35 gCOD/L*d when the amounts of both total VFAs and Acetic acid are maximum in the treated effluent and there is also a significant amount of Propionic acid.

Unlike the organic acids productivity and yield on substrate, the organic acids distribution is strongly affected by pH. Acetate and propionate concentrations decrease with decrease of pH from 7 to 5, while butyrate and valerate concentrations significantly increase for the same pH range [8].

Due to this fact, one of the specific objectives of this study was appointed to check if at higher operating pH there was better production of shorter chain VFAs (mainly, group of interest – Acetic, Propionic, i-Butyric). For that purpose Phase 2 was split into two Phases of experiment (A and B) at the same OLR of 33 gCOD/L*d but with different values of inlet alkalinity: during Phase A the inlet alkalinity was equal to 3.6 $gCaCO_3$ and during Phase B – to 4.1 $gCaCO_3$ (on experimental result to be checked if it was enough to increase the pH up to 6, and, consequently, the amount of acids' types of concern).

The acids distribution during Phases A and B are depicted in Figure 6.7, in order to understand better the effect of changing the alkalinity of the feeding solution.

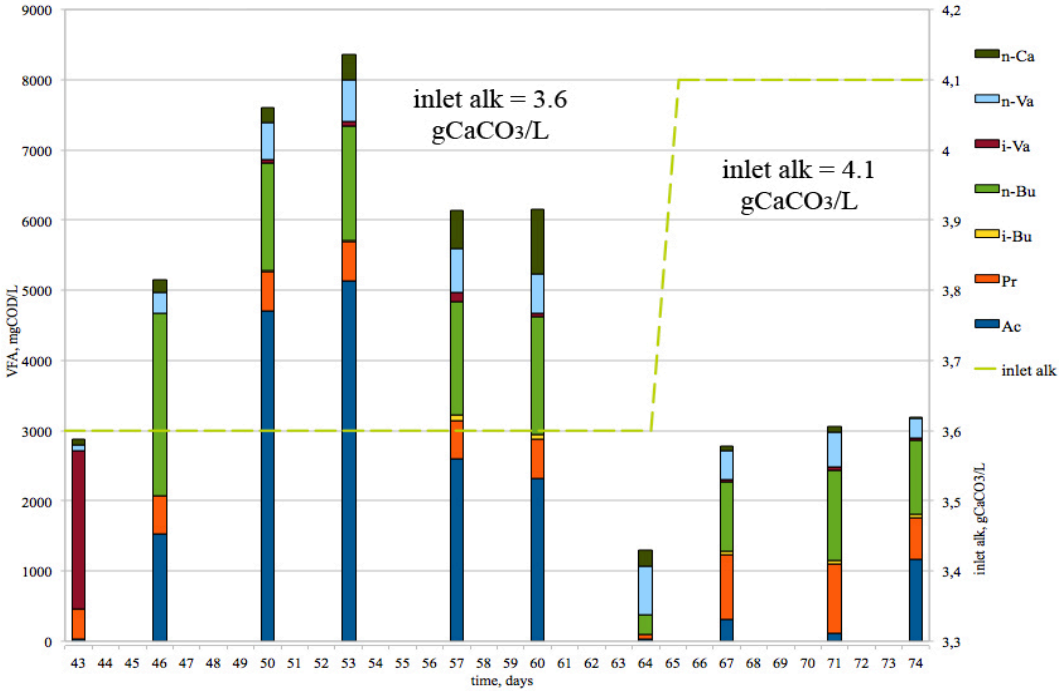


Fig. 6.7 – The VFAs' distribution during Phase A and B of Phase 2

On Figure 6.7 it can be easily seen that the increase on the inlet alkalinity from 3.6 $CaCO_3$/L (3.0 $gNaHCO_3$/L) to 4.1 $CaCO_3$/L (3.5 $gNaHCO_3$/L) at the same OLR = 33 gCOD/L*d leads to a decrease in the total VFA production and an increase in the amount of Propionic acid, as it was anticipated. However, it should be pointed out that the amount of Acetic acid dropped down dramatically. So, proportionally, the increase on the amount of Propionic acid is not comparable with the decrease on the amount of Acetic acid. The total amount of VFAs went down with the increase on the inlet alkalinity of less than a half value. So, it seems that the higher inlet alkalinity does not make a serious improvement on Propionic acid production. Moreover, it affects the Acetic acid production in a bad way.

To evaluate the effectiveness of the MBBR reactor performance it was necessary to consider the types of acids that belong to the group of interest – according to the specific objective for PHA production which are Acetic, Propionic and i-Butyric (2-Methylpropionic) ones. In Table 6.5 there are average data concerning these acids yield production including the data from the previous study – Phase 0 [15].

Tab. 6.5 – The Acetic, Propionic and i-Butyric acids' yield production

Phase #	(Acetic+ Propionic+ i-Butyric), % of total VFA	Type of acid, mg/L; % of total VFA			Total VFA average, mg/L
		Acetic	Propionic	i-Butyric	
Phase 0	69.6	2634	742	39	4909
		53.7	15.1	0.8	
Phase 1	46.8	660	272	311	2661
		24.8	10.2	11.7	
Phase 2A	62.7	2608	557	311	5541
		47.1	10.0	5.6	
Phase 2B	37.5	469	801	49	3517
		13.3	22.8	1.4	
Phase 3	28.5	743	1097	30.0	6561
		11.3	16.7	0.5	
Phase 4	64.8	3564	773	6.25	6706
		53.2	11.5	0.1	

In Table 6.5 is clearly seen that the highest amounts of acids of interest are allocated to three Phases: Phase 0 (70% at OLR = 21 gCOD/L*d); Phase 2A (63% at OLR = 33 gCOD/L*d); and Phase 4 (65% at OLR = 35 gCOD/L*d).

The Acetic acid was identified in high amounts at Phases 0 and 4 (53-54%). The lowest rate of acetate was determined at Phase 3 – only 11% (OLR = 66 gCOD/L) as well as the total amount of acids was the lowest at this phase – only 29%.

Looking at Phases A and B (Table 6.5) it is clearly seen that after changing the inlet alkalinity from 3.6 to 4.2 $gCaCO_3/L$ the total amount of VFAs dropped on 25%, decreasing from 63% to 38%; and, in turn, the amount of Acetic acid decreased on 34%.

The interesting situation with i-Butyric acid production was observed at Phase 1. Its average amount as percentage was almost 12%, however, according to Figure 6.7 it can be seen that there is no production of i-Butyric acid in the beginning at all. It suddenly appeared in a very high amount (up to 53% of total VFAs produced at Phase 1) only in

the end of the phase. At other phases i-Butyric acid was determined in very negligible amounts.

The propionate amounts were about 14% during Phases 0-2A and 3-4, except for Phase 2B where it was an incentive to check if the Propionic acid increases at higher alkalinity. It increased insignificantly – on 9% – up to almost 23%.

In order to assess the total amount of these acids produced, it should be considered the total amount of VFAs. The total amount of VFAs can be related to the COD_{out} or COD_{in} – Table 6.6.

Tab. 6.6 – The COD_{VFA} ratios to COD

Phase #	Average values of COD_{VFA} ratios	
	COD_{VFA}/COD_{out}, %	COD_{VFA}/COD_{in}, %
0	43.5	33.2
1	21.5	16.9
2A	28.8	21.8
2B	20.5	16.0
3	17.4	13.8
4	34.2	26.9

According to Table 6.6 the highest VFA yield production is obtained at Phase 0 (referring the previous study) and Phase 4: 44% and 33% (Phase 0), 34% and 27% (Phase 4), respectively, regarding COD_{out} or COD_{in}.

The best results, either in terms of total VFAs production or in terms of the important acids, are the ones obtained in the previous study (Phase 0) where the OLR was lower (21 gCOD/L*d) than the ones studied in this experiment and presented in Tables 6.5 and 6.6.

The following figure – Figure 6.8 – presents data of VFAs distribution inside the rector (Sampling point #3 – Figure 4.1).

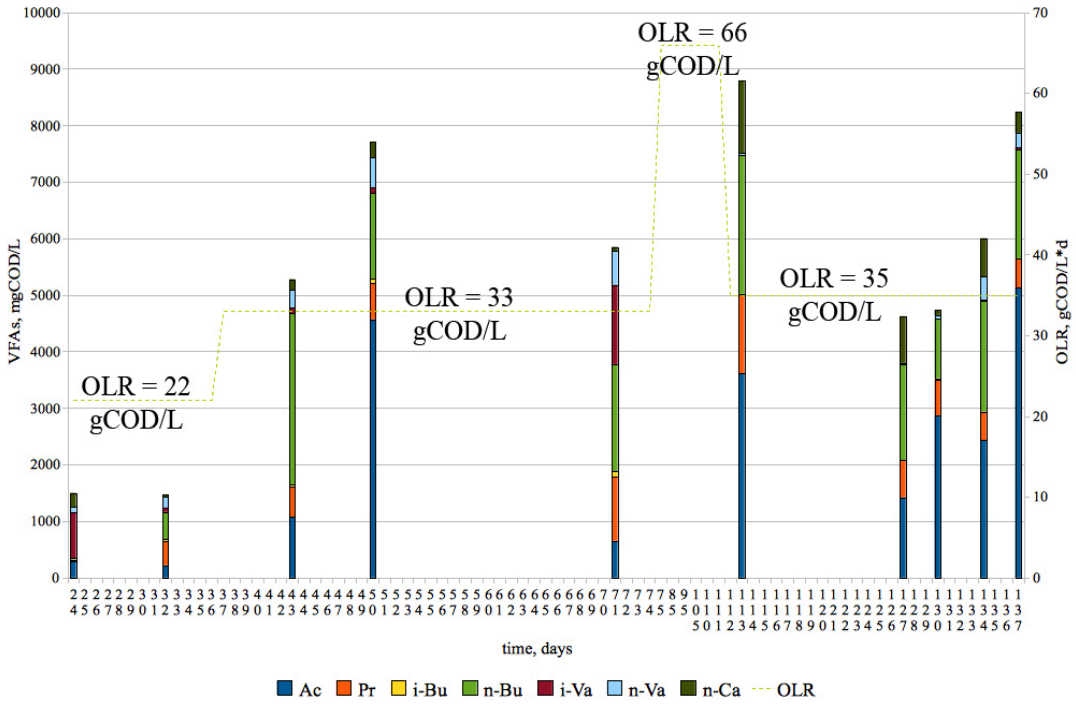


Fig. 6.8. – The VFAs' distribution inside the reactor

According to the Figure 6.8. it is possible to conclude that the individual VFAs are distributed similarly to the ones obtained from the analyses taken from the clarifier (Sampling point #2 – Figure 4.1) at correspondent phases. The main constituents are Acetic, n-Butyric and Propionic acids. The rest of acids either appear in very small amounts or do not appear at all. At Phase 3 (OLR = 66 gCOD/L) there is no data because sampling collection was not possible.

6.3 Biogas production

To measure the biogas production for each operational condition it was used a gas measuring device – the precision wet-test gas flow meter of drum-type RITTER® TG 05.

The equipment to measure the main the main components of biogas (CO_2 and CH_4) was a gas chromatograph SRI 8610C with TCD detector.

The other quite important parameter to which the attention was paid was the composition of the biogas which was produced during the acidification process – Figure 6.9.

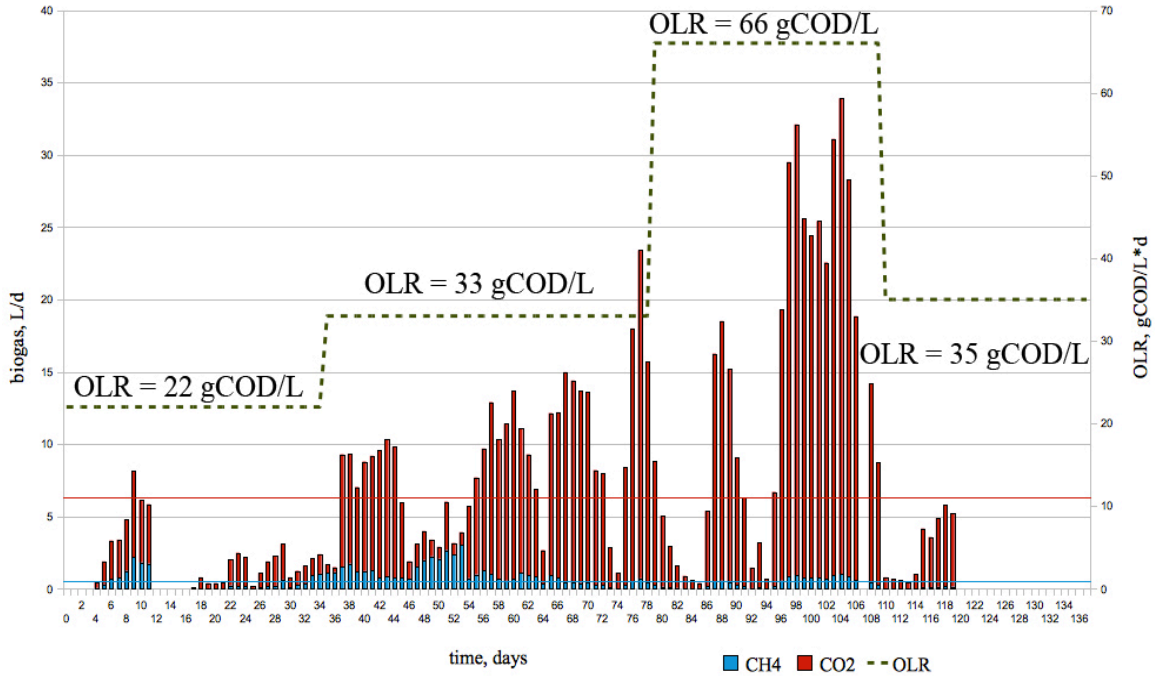


Fig. 6.9 – The total amount of biogas and its main components (CO_2 and CH_4)

According to Figure 6.10 the production of CH_4 is very low and varies from 0.0 to 3.0 L/d.

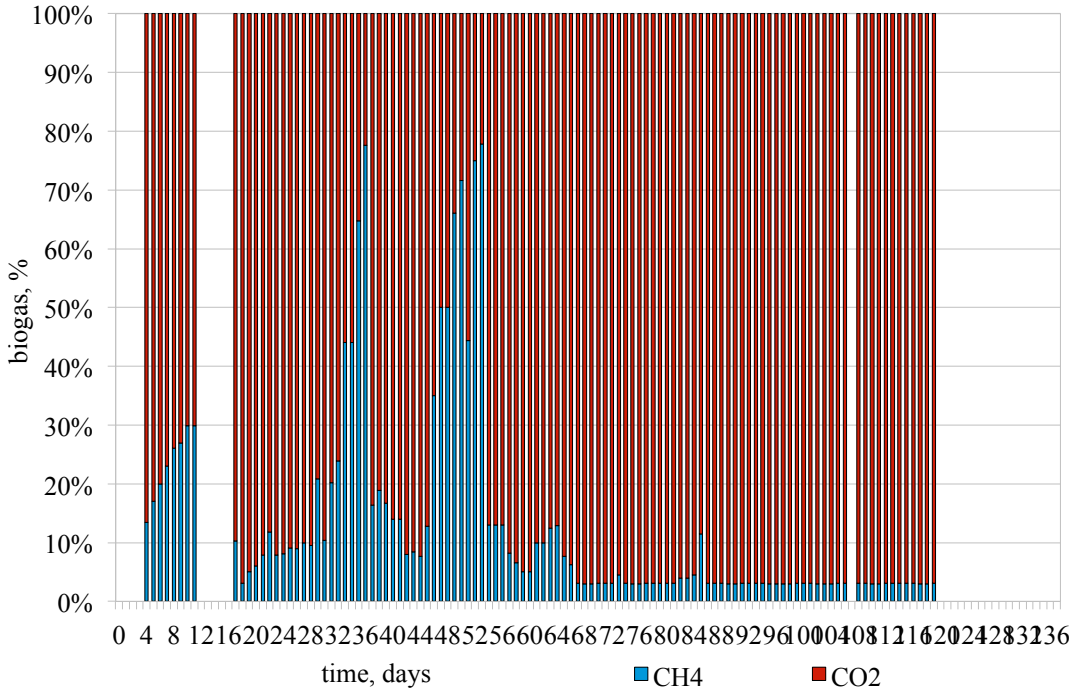


Fig. 6.10 – The main biogas components (CO_2 and CH_4) as 100% distribution

As it can be seen in Figures 6.9 and 6.10 the amount of CH_4 varies a lot and has a tendency to increase during the initial stages of the experiment, which is not a good sign for VFAs production. However, it keeps quite high only for a short period of time. It has a cyclic character. Then, during Phase 2, its level dramatically drops down after 52-56 days of experiment until it reaches the point of zero and stays like that until the end of experiment.

As it is known the methanogenic bacteria are the ones who are more prone to be influenced by the change in the environmental conditions, so they, apparently, were affected by the low pH, a high level of VFAs and increasing OLR.

To analyse in detail the dynamics of these two figures it is important to consider the following graphs in Figures 6.11-6.14.

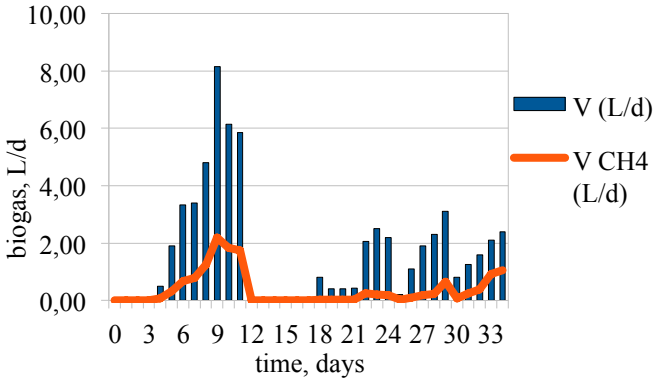


Fig. 6.11 – CH_4 distribution during the 1st time period of experiment

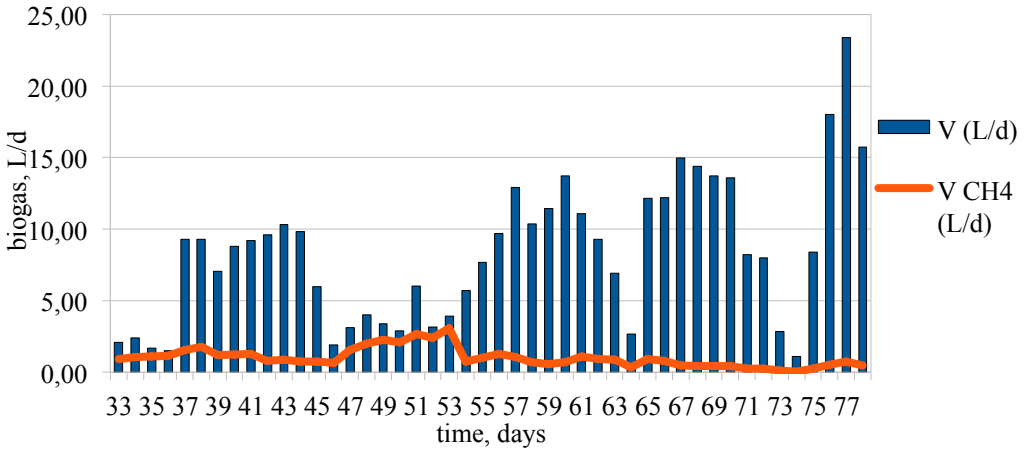


Fig. 6.12 – CH_4 distribution during the 2nd time period of experiment

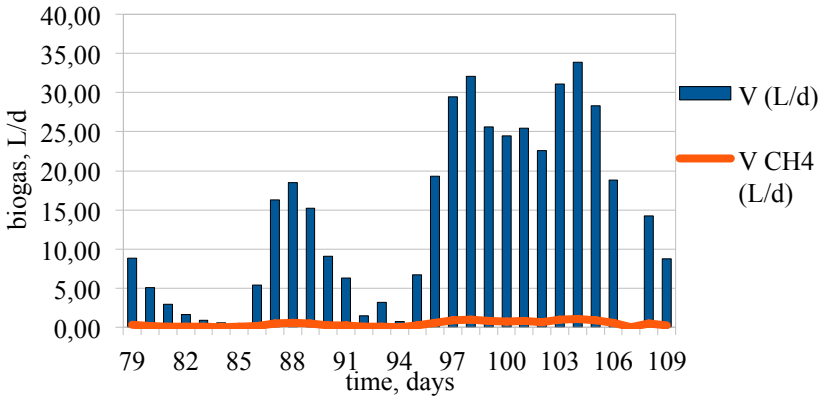


Fig. 6.13 – CH_4 distribution during the 3rd time period of experiment

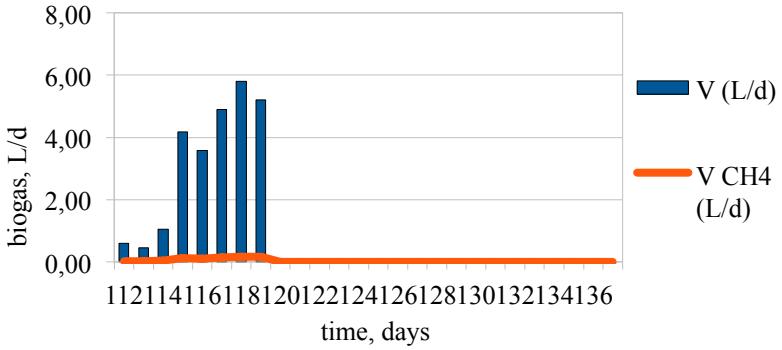


Fig. 6.14 – CH_4 distribution during the 4th time period of experiment

According to Figures 6.11-6.14 the average values for each period of experiment are presented in Table 6.7. It can be seen that the total biogas increases with the OLR, whereas with respect to methane it increases until Phase 2 (OLR = 33 gCOD/L*d) and drops afterwards to very low values.

Tab. 6.7 – The average values of total biogas and CH_4 amounts

Phases of experiment	Average value	
	Total biogas, L/d	CH_4, L/d
Phase 1	2.3	0.52
Phase 2	8.3	1.01
Phase 3	12.7	0.39
Phase 4	3.2	0.10

According to Figure 6.11 which represents the results of methane production during the 1st phase of experiment, it can be seen that the amount of CH_4 is not high – maximum 2 L/d.

During some days (12-17) there is no biogas data because the calibration of the feeding pump was conducted. The methane production, as well as biogas production in general, has a cyclic character due to a tendency to increase when there was a new feed preparation and decline afterwards.

Looking at Figure 6.12 it is possible to conclude that on some days the amount of methane emitted is disproportionately low towards all biogas produced, and on other days methane production increases proportionally to biogas. Apparently, that happened due to the non-stable conditions of the system. After the 55th day, probably when the steady state was reached, the CH_4 emission starts to decrease constantly until a very low value. It can be explained in two ways. Either the carriers to which the bacteria stick with are not sufficient to protect the methanogenic bacteria from any influence from outside, so they stop producing methane, or the OLR = 33 gCOD/L*d is already too high which leads to overloading the methanogenic biomass capacity. Generally, both these reasons could contribute to such a result. In addition, problems with the mixer could deteriorate the conditions inside the MBBR reactor.

On Figure 6.13 the results from Phase 3 depicted where the applied OLR = 66 gCOD/L*d. There is no surprise that for this load the level of methane production is very insignificant or even almost equal to 0. Despite this, the general amount of biogas emitted is cyclic and from time to time increases and drops down very sharply.

The 4th period of experiment – Figure 6.14 – which was conducted after cleaning the MBBR reactor and fixing the mixer cannot be characterised completely as it seems that some monitoring equipment was malfunctioning starting since the 119th day. But according to the gas chromatography data the amount of methane first decreased until it became negligibly small. It can be explained due to the fact that after the MBBR reactor cleaning about 40% of its biomass content was put back. The previous OLR was equal to 66 gCOD/L*d which might specify the biomass in the reactor for mainly acids production microorganisms.

6.4 Biomass evaluation

6.4.1 Quantification of biomass

Figure 6.15 represents the results of TSS and VSS analyses. The samples were taken from both the clarifier and reactor to evaluate the growth of biomass.

TSS analysis shows the concentration of particulate material, and VSS analysis gives the organic matter in a sample corresponding to the biomass, as the amount of particulate matter in the feed is negligible.

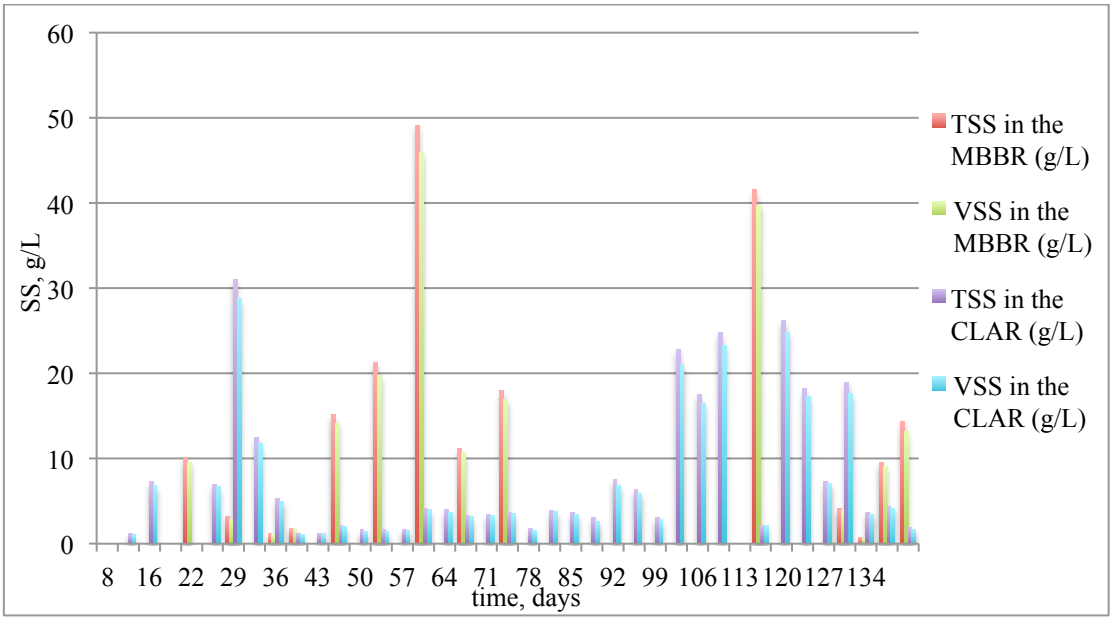


Fig. 6.15 – The concentration of TSS & VSS in the MBBR reactor and clarifier vs. time

As it can be seen, the amount of TSS varies a lot, either inside the reactor or in the clarifier. However, the ratio between VSS and TSS stays constant and approximately around 94% – according to the data in Table 6.8. The VSS is quite a big part of TSS, which is good. It shows that most of the suspended solids are biomass, due to the fact that the suspended matter in the feed is negligible. The lack of analyses taken from the reactor during Phase 3 was due to the difficulty in withdrawal of a sample from the reactor because of its clogging.

Although average values for the whole period of experiment vary a lot, most of values are lower than 10 g/L of TSS. Due to this fact they seem to be reasonable to be presented (Table 6.8).

Tab. 6.8 – The average values of TSS and VSS data according to Figure 6.14

Parameter	TSS in the MBBR, g/L	VSS in the MBBR, g/L	TSS in the CLAR, g/L	VSS in th CLAR, g/L
	14.4	13.5	7.7	7.2
VSS/TSS ratio, %	93.8		93.5	

In the following figures there are data of TSS and VSS in the MBBR reactor and

clarifier within the time frameworks of each phase – Figures 6.16 and 6.17.

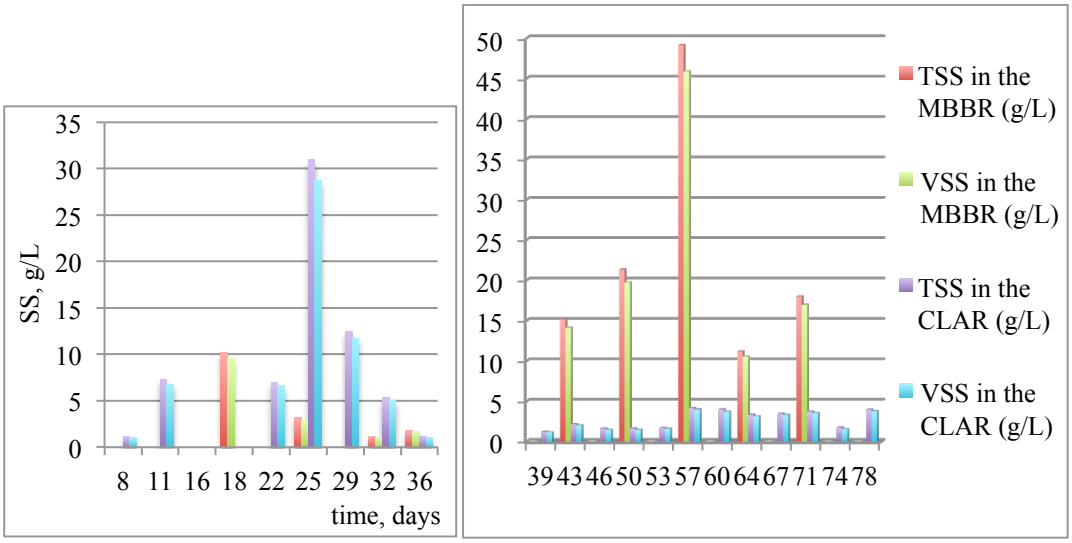


Fig. 6.16 – TSS and VSS concentration during the 1st (left) and 2nd (right) phases of experiment

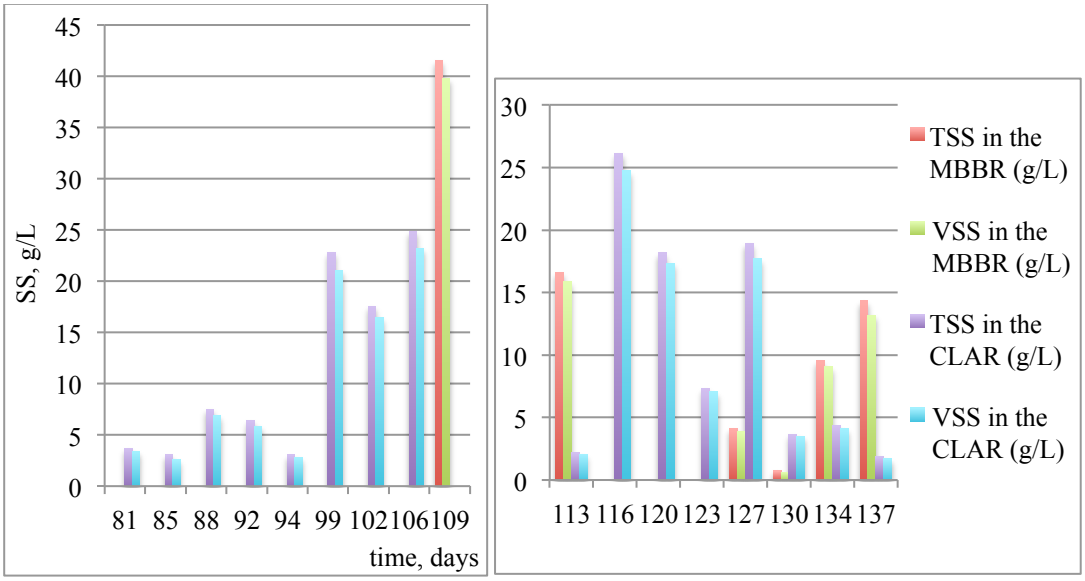


Fig. 6.17 – TSS and VSS concentration during the 3rd (left) and 4th (right) phases of experiment

When considering Figure 6.16 and the left graph it can be seen that the amount of TSS and VSS inside the clarifier increases, which indicates the reactor performance with respect to suspended solids (when it retains on discharged solids). At the first OLR applied, the bacteria are growing but they are not yet all completely attached to the carriers inside the reactor, so, they are being washed out to the clarifier.

Analysing the right picture on Figure 6.16, after increasing an OLR up to 33 gCOD/L*d, the biomass which is 93% of all organic particulate matter inside of the MBBR start growing much more and accumulating in the reactor (due to the carrier attachment). Although in the reactor there is a high amount of biomass (more than 15 gTSS/L), in the clarifier this amount is very insignificant (less than 5 gTSS/L), which is, probably, due to the biomass attachment to the carriers and retention.

Apparently, because of high retention and mixer malfunctioning occurrence during Phase 2, the biomass had been accumulating inside the reactor until the moment when it turned out impossible to collect a sample from the MBBR (Phase 3). So, Figure 6.17 (left side) presents only the results of biomass dynamics in the clarifier when the OLR = 66 gCOD/L*d. The levels of TSS and VSS had been increasing until they reached very high values (22-25 gTSS/L), leading to the conclusion that the reactor was completely overloaded. Hence, during that phase it was not possible to measure the biomass inside the reactor. The last measurement pointed out in the graph was done when the reactor was opened, and the mixing system was fixed.

After opening the reactor with the purpose of its clean up and maintenance of the mixer, the mixed liquor analysis showed that the TSS was up to 40 gSS/L. As afterwards about 40% of total volume of mixed liquor, used during Phase 3, was put back into the reactor, it allowed to conclude that the initial concentration of TSS inside the reactor was about 16 gSS/L. So, on the Figure 6.17 (right side) it can be seen that after starting the reactor the amount of TSS and VSS in the clarifier decreased gradually and after a drop down from 16g/L to almost zero (days 113-127) started to increase inside the reactor.

It is important to mention that the speed of mixing was constant during the whole experimental time, except for a few days in the middle of 4th phase that had an objective to see if it influences the SS dynamics. During the period since day 123rd until day 127th the mixer was at a higher speed (8th speed at the range 1) which corresponded to 260 rpm. It resulted in washing out of more particulate matter from the reactor into the clarifier (Figure 6.6, right side). Therefore, the speed was put back to the 5th speed at the range 1 (130 rpm).
For better understanding of the biomass growth behaviour, in Tables 6.9 and 6.10 are presented the average data from Figures 6.16 and 6.17 which show amounts of TSS and

VSS for each phase and also its ratios VSS/TSS in both MBBR reactor and clarifier.

For Phase 3 (concerning Tables 6.9 and 6.10), the results given are based on analyses performed only after opening the reactor, as it was not possible to take a sample from the MBBR reactor during this phase because of its organic overload.

Tab. 6.9 – The average values of TSS and VSS data

Phases	Average value			
	VSS/TSS ratio in the MBBR, %		VSS/TSS ratio in the CLAR, %	
	TSS in the MBBR, g/L	VSS in the MBBR, g/L	TSS in the CLAR, g/L	VSS in the CLAR, g/L
Phase 1	4.1	3.8	9.3	8.6
	92.5%		91.8%	
Phase 2	22.9	21.4	2.5	2.4
	93.5%		93.7%	
Phase 3	41.5	39.8	11.1	10.9
	95.7%		98.5%	
Phase 4	9.1	8.5	10.3	9.8
	94.0%		94.6%	

According to Table 6.9 it can be seen that despite the high variations of the amounts of TSS and VSS during Phases 1-4, the ratios of VSS to TSS are kept almost the same. In the MBBR reactor VSS/TSS ratio varies a little – from 92.5% to 95.5%, and in the clarifier it varies a bit more – from 92.0% to 98.5%. It can also be seen that TSS and VSS increase with the increase of OLR (Phase 1 to 3) and decrease with the decrease of OLR (Phase 4).

Phases 2 and 4 present very different solids content, although they present the same organic load. It happened because it was necessary to restart the reactor, and the biomass did not have enough time to attach in big scale to the carrier material.

The following table – Table 6.10 – reflects the ratios of TSS and VSS in the clarifier and MBBR reactor

Tab. 6.10 – The ratios of TSS and VSS in the clarifier (CLAR) and MBBR reactor

Phase #	Ratio between	
	TSS in the CLAR and MBBR (TSS_{CLAR}/TSS_{MBBR}), %	VSS in the CLAR and MBBR (VSS_{CLAR}/VSS_{MBBR}), %
1	228.0	226.2
2	11.0	11.0
3	26.7	27.5
4	113.9	114.7

In Table 6.10 it is possible to notice that TSS_{CLAR}/TSS_{MBBR} and VSS_{CLAR}/VSS_{MBBR} vary a lot: they are higher at lower OLR (Phases 1 and 4) and lower at higher OLR (Phases 2 and 3). Hence, the conclusion is that the proper MBBR performance can be observed at TSS_{CLAR}/TSS_{MBBR} and VSS_{CLAR}/VSS_{MBBR} higher than 100%, and at TSS_{CLAR}/TSS_{MBBR} and VSS_{CLAR}/VSS_{MBBR} values lower than 50% the MBBR reactor does not present its proper performance.

With these results it can be said that the MBBR reactor works properly with a mixed liquor biomass content between 5 and 10 g/L of TSS inside the reactor and about 10 gTSS/L in the clarifier, at a moderate speed of mixing (130 rpm), as it can be seen in the final part of Phase 4.

6.4.2 Microscopic evaluation

To see what types and structures of microorganisms inside the reactor some picture were taken with a help of microscope ZEISS Imager.A2 equipped with the camera Axio Cam MRm (Figure 6.18).

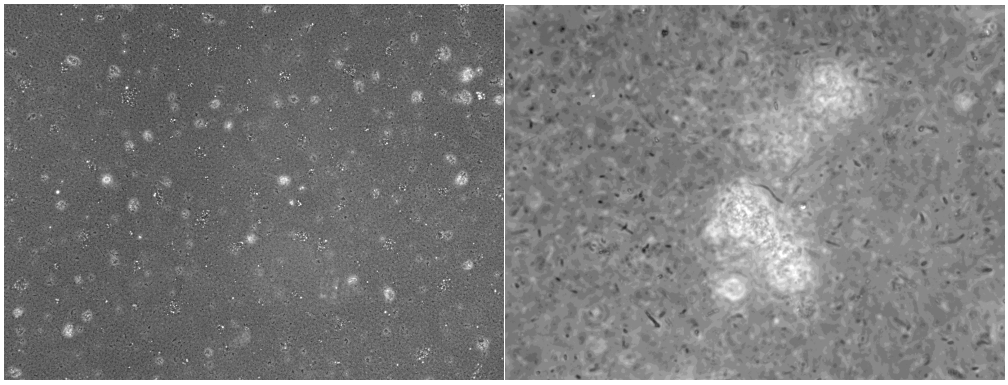


Fig. 6.18 – The photos of the content of MBBR reactor at the OLR = 33 gCOD/L*d, magnified in 10 and 100 times, respectively

The photo depicted on Figure 6.18 was taken of a sample collected from the MBBR during Phase 2.

Looking at Figure 6.18 it is possible to say that at an OLR of 33 gCOD/L*d (Phase 2) there are few granules and with a small size and reduced density. The granules are mainly composed of cocci shape bacteria. Bacteria outside the granules appear in cocci and bacilli shapes. Some bacilli shape bacteria appear in filamentous colonies. The bacteria associated with the granules are not identifiable.

In the end of Phase 3 after opening the reactor with the purpose of its cleaning the analysis showed that the TSS and VSS were up to 40 g SS/L. It was then possible to have some observations over the samples from the mixed liquor and the carriers with attached biomass. Figure 6.19 shows the biomass in the mixed liquor and carriers after Phase 3.

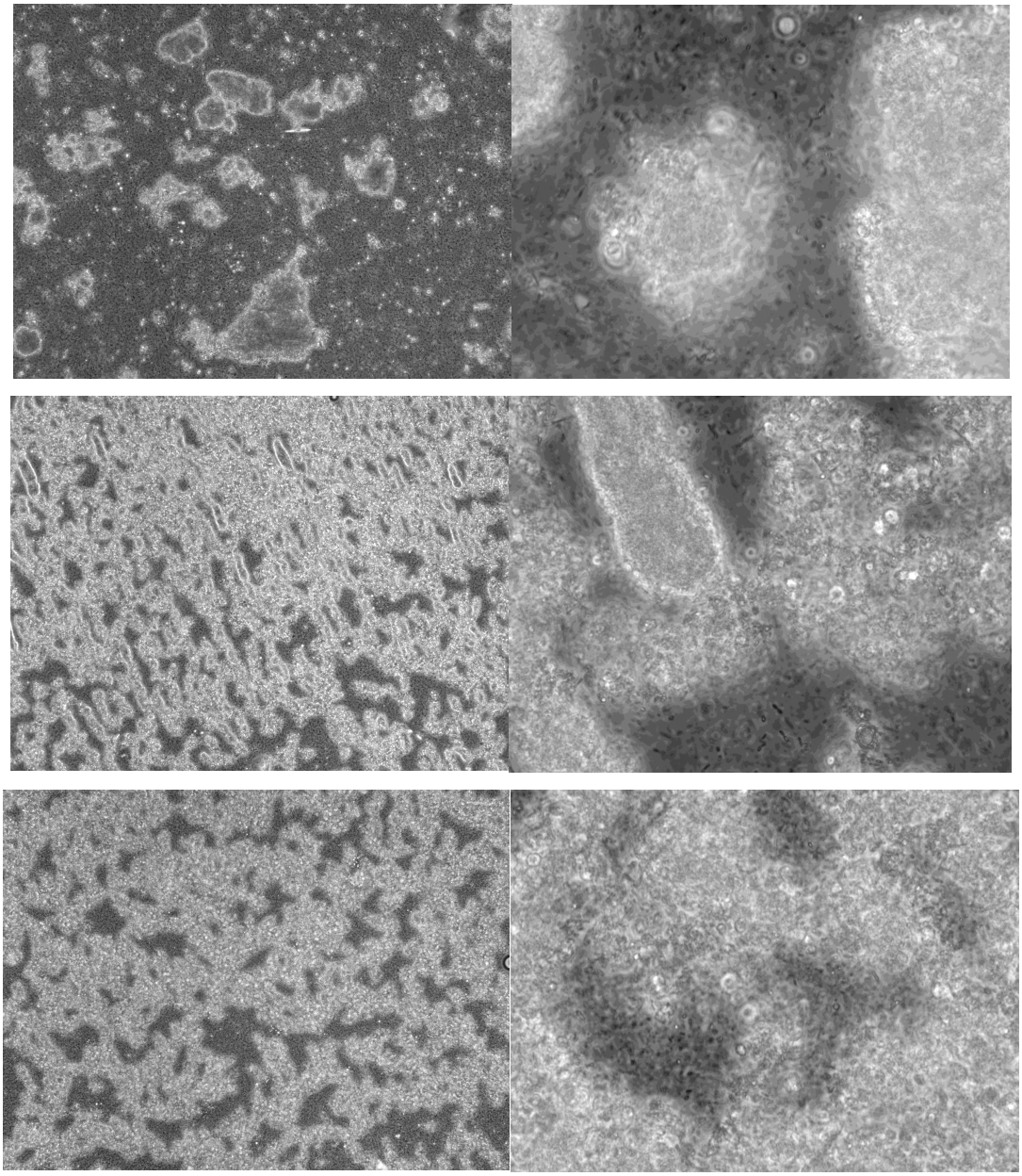


Fig. 6.19 – The photos of the content of MBBR reactor (upper photos – sample of mixed liquor suspended solids; middle photos – sample taken from the outer side of carriers; lower photos – sample taken from the inner side of carriers) after reactor opening (when the OLR = 66 gCOD/L*d), magnified in 10 and 100 times, respectively

The biomass inside the carriers is very dense. The granules are all connected between themselves, forming an almost continuous mass. The bacteria associated with the granules are also not identifiable. Bacteria outside the granules appear in cocci and bacilli shapes. Some bacilli shape bacteria appear in filamentous colonies.

The biomass outside the carrier is also very dense, but slightly lesser than the biomass

inside. There are unique granules with cylindrical shapes that are not present in any other location. To identify the bacteria associated with the granules is difficult. The shapes of the bacteria outside the granules are similar to the other samples but the filamentous colonies consist of maximum two bacilli shape bacteria.

The suspended biomass in the mixed liquor has separated granules (not connected between themselves) and a lot of suspended bacteria. The granules are very dense which makes the bacteria identification difficult. The granules also appear thicker than the ones presented in the previous samples taken at lower organic load (33 gCOD/L*d). Bacteria outside the granules appear in cocci and bacilli shapes. Some bacilli shape bacteria appear in filamentous colonies.

Samples were also taken from the MBBR reactor, but only from the mixed liquor at the OLR = 35 gCOD/L*d. The pictures from microscopic analysis are in Figure 6.20.

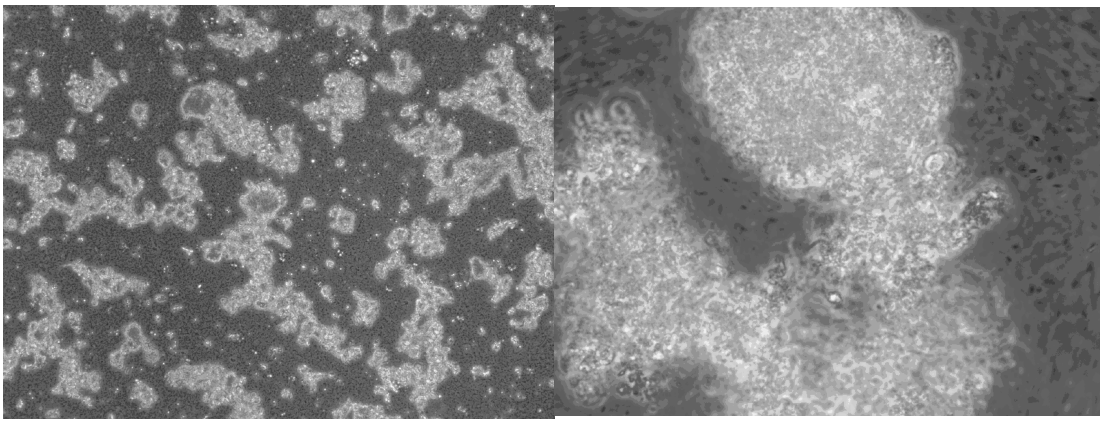


Fig. 6.20 – The photos of the content of MBBR reactor at the OLR = 35 gCOD/L*d, magnified in 10 and 100 times, respectively

Inside the MBBR reactor in the mixed liquor there are many granules, with variable sizes and densities. The bigger granules are almost connected between themselves. Bacteria outside the granules also appear in cocci and bacilli shapes. Some bacilli shape bacteria appear in filamentous colonies, mainly composed only by two species.

6.5 The mass balance of the system

In order to understand better the performance of the MBBR reactor at the different operational conditions tested, a mass balance of the system was done for each phase. It required some calculations based on data obtained and treated from analyses and data resulting from theoretical considerations. Hence, Table 6.11 was built, where four calculating steps were considered and all phases were described according to the studied parameters of the system.

Tab. 6.11 – The mass balance of the system

		1st step: Feed data average COD_{in} (mg/L)		2nd step: MBBR + Biogas data average COD_r + COD_{bio} + COD_{CH4} (mg/L)					3rd step: CLAR + Biogas data: average COD_{out} + COD_{bio} + COD_{CH4} (mg/L)				4th step: CLAR + Biogas data: average COD_{VFA} + COD_{other} + COD_{bio} + COD_{CH4} (mg/L)			
	conditions: OLR (gCOD/L), alkalinity, ($gNaHCO_3$/L)	COD_{in}		COD_r (1)	COD_{bio_dif} (2)	COD_{bio_calc} (3)	COD_{CH4} (4)		COD_{out} (5)	COD_{bio} (6)	COD_{CH4} (7)		COD_{VFA} (8)	COD_{other} (9)	COD_{bio} (10)	COD_{CH4} (11)
Phase1	OLR=22, alk=3	15729		14943	-619	13657	1405		12351	7636	1405		2661	9690	7636	1405
				COD_{sum1} = (1) + (2) + (3) + (4) =			21725		COD_{sum2} = (5) + (6) + (7 =		21392		COD_{sum3} = (8) + (9) + (10) + (11 =			21392
Phase2	OLR=33, alk_{max}=3.5	24262		27173	-5281	55317	2370		18621	3723	2370		4918	13703	3723	2370
				COD_{sum1} = (1) + (2) + (3) + (4) =			59995		COD_{sum2} = (5) + (6) + (7 =		24714		COD_{sum3} = (8) + (9) + (10) + (11 =			24714
Phase3	OLR=66, alk=3	47469		*	*	*	967		37795	6428	967		6561	31234	6428	967
							*		COD_{sum2} = (5) + (6) + (7 =		45190		COD_{sum3} = (8) + (9) + (10) + (11 =			45190
Phase4	OLR=35, alk=3	24927		22684	1928	18877	315		19631	4042	315		6706	12925	4042	315
				COD_{sum1} = (1) + (2) + (3) + (4) =			41876		COD_{sum2} = (5) + (6) + (7 =		23988		COD_{sum3} = (8) + (9) + (10) + (11 =			23988

*	-	no data due to the difficulty in collecting samples from the MBBR reactor

The 1st step corresponds to the results obtained in the analyses for average COD_{in} for each phase conducted over samples taken from cheese-whey feeding solution (Sampling point #1 – Figure 4.1).

The 2nd step corresponds to the average data values of COD_r and COD_{bio} (for samples taken from the MBBR reactor – Sampling point #3 – Figure 4.1) and COD_{CH4} (for samples taken in the biogas amount). The COD_r was determined by performing the sCOD analysis, and COD_{bio_calc} and COD_{CH4} were calculated according to the theoretical Equations 5.1, 5.2. COD_{bio_dif} was obtained by subtracting from COD_{out} the sum of COD_r and COD_{CH4}: $COD_{bio\_dif} = COD_{out} - (COD_r + COD_{CH4})$. These two methods were performed in order to better evaluate the bacterial growth and the MBBR reactor efficiency in general.

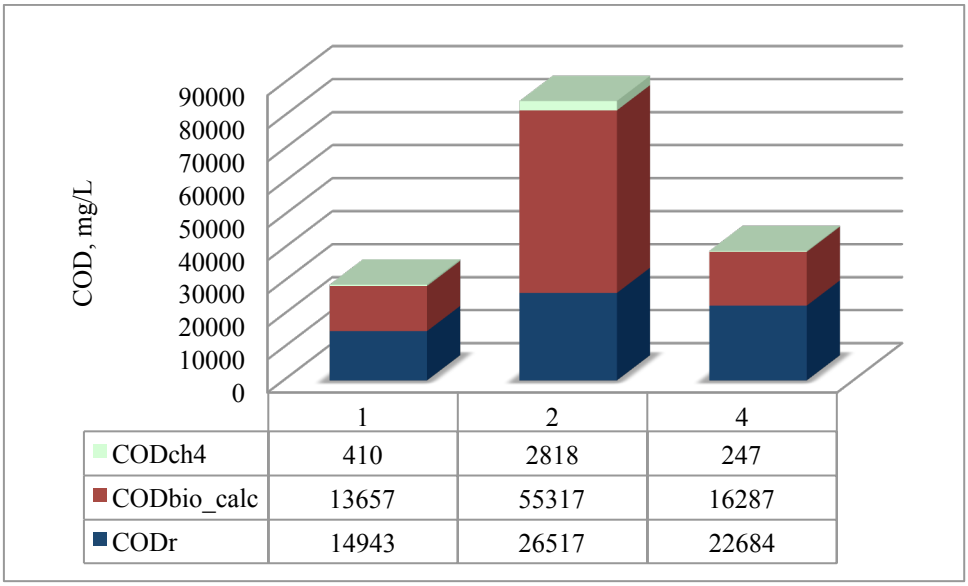
The 3rd and the 4th steps represent the results obtained from the analyses performed over the effluent from the clarifier (Sampling point #2 – Figure 4.1) and biogas amount. COD_{out} was determined by the sCOD analysis, whereas COD_{CH4} and COD_{bio} were computed according to Equations 5.1, 5.2, respectively, similarly to what was done in the 2nd step of the mass balance.

In the 4th step of the mass balance, the split of COD_{out} of treated effluent into the two components – COD_{VFA} and COD_{other} – shows how much of COD_{out} corresponds to the production of VFAs and how much corresponds to other constituents. The computations of COD_{VFA} were performed using Equation 5.3. COD_{other} was obtained by mathematical difference: $COD_{other} = (COD_{out} - COD_{VFA})$.

It is necessary to emphasise that the clarifier is an open unit, hence, there is no biogas measurement. However, it was realised that some biodegradation occurred in the clarifier, which played a role of reactor within the time between two sampling days (sampling was performed twice per week). Therefore, the data of COD_{CH4} presented in steps 2-4 are the same, and referred to the measurements of biogas amounts from a gas flow meter connected to the MBBR reactor.

Figure 6.21 presents the 2nd step of Table 6.11 where it can be seen the different proportions of COD_r, COD_{bio_calc} and COD_{CH4} in the MBBR reactor resulting from the

organic matter present in feeding solution for each phase of experiment.



	1	2	4
CODch4	410	2818	247
CODbio_calc	13657	55317	16287
CODr	14943	26517	22684

Fig. 6.21 – The data from the 2nd step of the mass balance scheme

In the next figure – Figure 6.21 – there are data on average COD_r + COD_{bio_calc} + COD_{CH4} (2nd step of Table 6.11).

In this figure, the biomass growth was correspondent to COD_{bio_calc} obtained from the conversion of the VSS measured to COD (Equation 5.2).

It can be seen that during the 1st phase the microorganisms were not yet all attached to the carriers, and its surplus was washed out from the reactor to the clarifier (COD_{bio_calc} value of Phase 1 is the lowest within this step of the mass balance).

From this mass balance (2nd step), it can be seen that the COD_r in Phase 2 is higher than in Phase 4. Hence, it can be said that during the 2nd phase the biomass attached to the carriers and the surplus of it started to be trapped inside of the reactor which led to the biomass accumulation and reactor overload (COD_{bio_calc} reached around 55g/L as COD). Hence, COD_{bio_dif} gave a negative value of approximately 5.3 g/L as COD. This shows the initial stage of accumulating problem which appeared in bigger scale during Phase 3 (that forced the opening and cleaning up of the reactor).

There is no data for Phase 3, by reason of the fact that there was no possibility to collect

samples from the MBBR reactor.

During Phase 4 after reactor opening and cleaning and mixer fixing up, the situation depicted approaches the one of Phase 1. The main difference, apart from different OLRs applied, is that the biomass must be better attached to the carriers. Comparing to Phase 2, the biomass is not trapped inside the reactor. The COD_r value in Phase 4 better reflects the normal reactor performance.

Figure 6.22 – shows the relation between the COD_{in} and the sum of different COD components considered as COD_{sum1}, which consists of $COD_r + COD_{bio\_calc} + COD_{CH4}$.

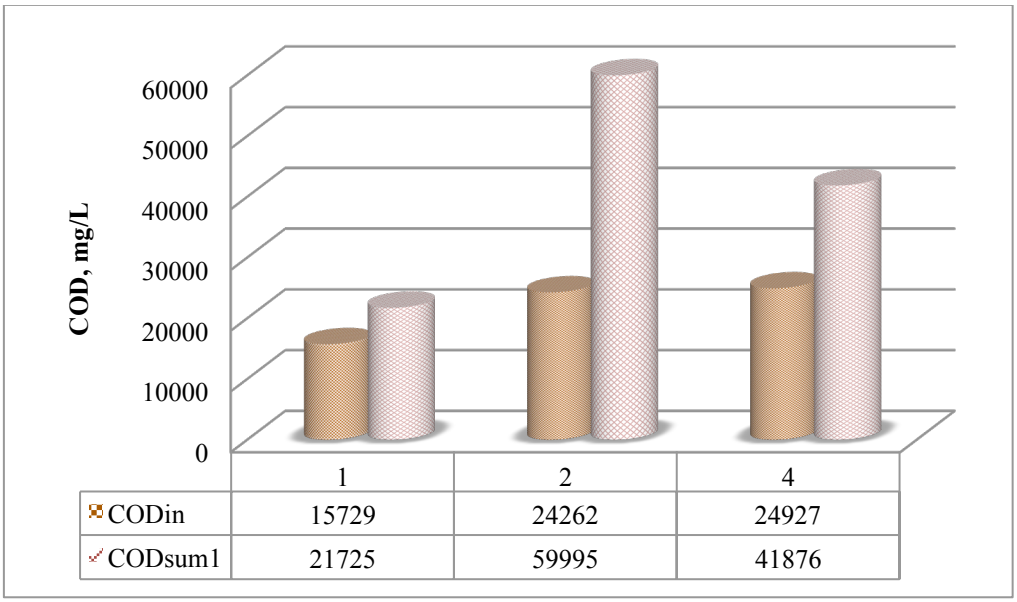
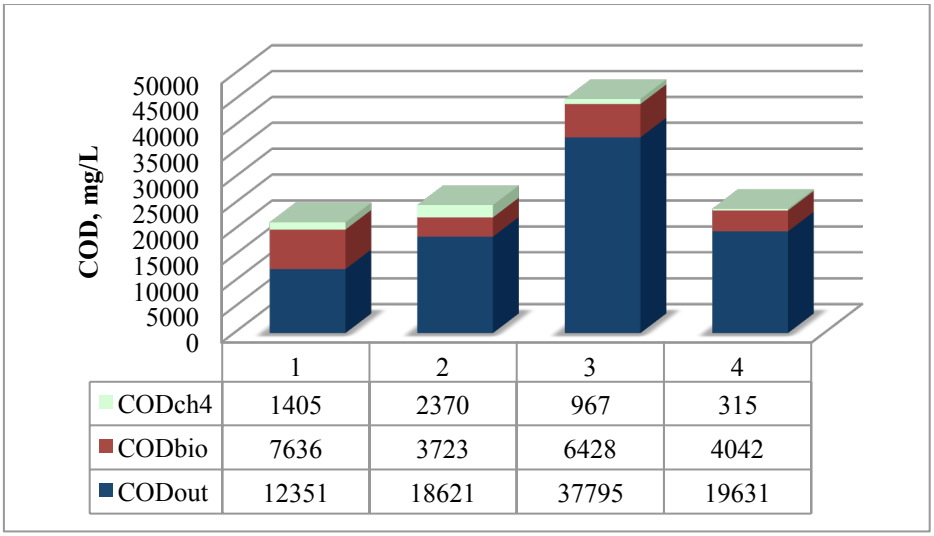


Fig. 6.22 – The relation between the COD_{in} and COD_{sum1} (2^{nd} step)

According to Figure 6.22 it is possible to conclude that during Phases 1 and 4, when the COD_{in} approaches COD_{sum1}, the biomass is not yet completely attached (Phase 1 – at the start of MBBR reactor running; Phase 4 – after opening the reactor and disturbing the content inside it) and washed out a lot, so, apparently, there is a lot of organic matter (COD_r) and not enough bacteria grown to consume that load. During the 2^{nd} phase that high increase of biomass reflects in the amount of bacteria which are already attached to the carriers and trapped inside the reactor (COD_{bio_calc} reached very high values) due to the increase of OLR from 22 to 33 gCOD/L*d. Hence, as a result there is a plenty of biomass which contributes to high COD_{bio_calc} values and, consequently, to high COD_{sum1} values. This situation leads to the reactor overload and malfunction. Therefore,

the way of biomass growth measurement (sampling from the MBBR reactor) was not good for the mass balance, due to the fact that that measurement accounted for the biomass growth plus the biomass inside the reactor.

Figures 6.23, 6.24 and 6.25 were depicted according to the Table 6.11 as well for the 3rd and 4th steps.



	1	2	3	4
CODch4	1405	2370	967	315
CODbio	7636	3723	6428	4042
CODout	12351	18621	37795	19631

Fig. 6.23 – The data from the 3rd step of mass balance scheme

Figure 6.23 presents the data from the 3rd step of the mass balance described in Table 6.11. The COD_{bio} data were calculated according to analyses taken from the clarifier, which gave a completely different depiction of the situation towards the one obtained in the 2nd step of the mass balance.

There is no any regularity in changing COD_{bio} towards COD_{out}. Here, during Phase 1 the biomass growth had quite a high value (7636 mg/L as COD) due to the fact that the biomass was growing and washing out from the reactor (little attachment to the carriers). Then COD_{bio} decreased while COD_{out} increased: the OLR got higher (from 22 to 33 gCOD/L), which led to higher values of COD_{out}, and, simultaneously, the attachment of biomass to the carrier material became better due to the long time of reactor performance – more than 30 days (Phase 2). During Phase 2, COD_{bio} dropped down to 3723 mgCOD/L. As the biomass was accumulating inside the reactor (results from the 2nd step), only smaller amount reached the clarifier. Then, COD_{bio} increased up to 6428 mg/L as COD with a sharp increase of COD_{out} from 18621 to 37795 mgCOD/L:

at higher OLR (an increase from 33 to 66 gCOD/L) the reactor started becoming overloaded, which caused the wash out from the MBBR reactor and, consequentially, the higher average COD_{bio} value of effluent in the clarifier (Phase 3). At Phase 4, both parameters COD_{bio} and COD_{out} decreased by the reason of cleaning the reactor, fixing the mixer and lowering the OLR (from 66 to 35 gCOD/L).

This COD_{bio} value (4042 gCOD/L) represents the biomass growth and demonstrates the reactor performance when a constant amount of bacteria are attached to the carriers, and only the part of them which reflects the biomass growth is not washed out. Hence, reactor cleaning and mixer fixing up before Phase 4 resulted in reactor performance improvement.

At Phase 4, at OLR = 35 gCOD/L*d, COD_{bio} is almost equal to the one of Phase 2 (OLR = 33 gCOD/L*d), except data for CH_4. The total amount of biogas as well as the amount of CH_4 decreases because of the methanogenic microorganisms suppression by specific bacteria presented in the MBBR reactor after applying a very high OLR at Phase 3.

On Figure 6.24 is shown the relation between the COD_{in} and sum of different components considered COD_{sum2} which consists of COD_{VFA} + COD_{other} + COD_{bio} + COD_{CH4}.

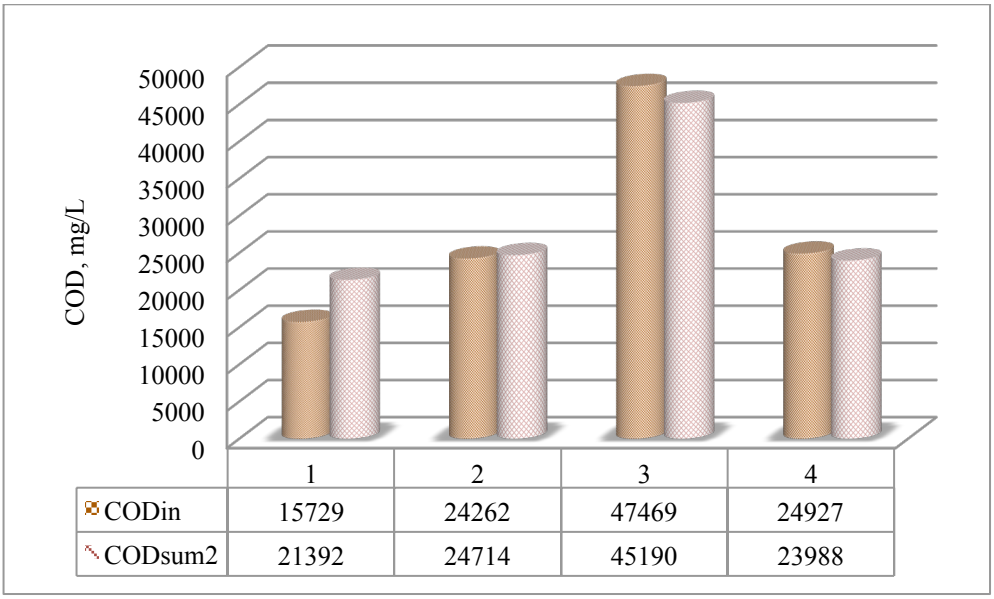
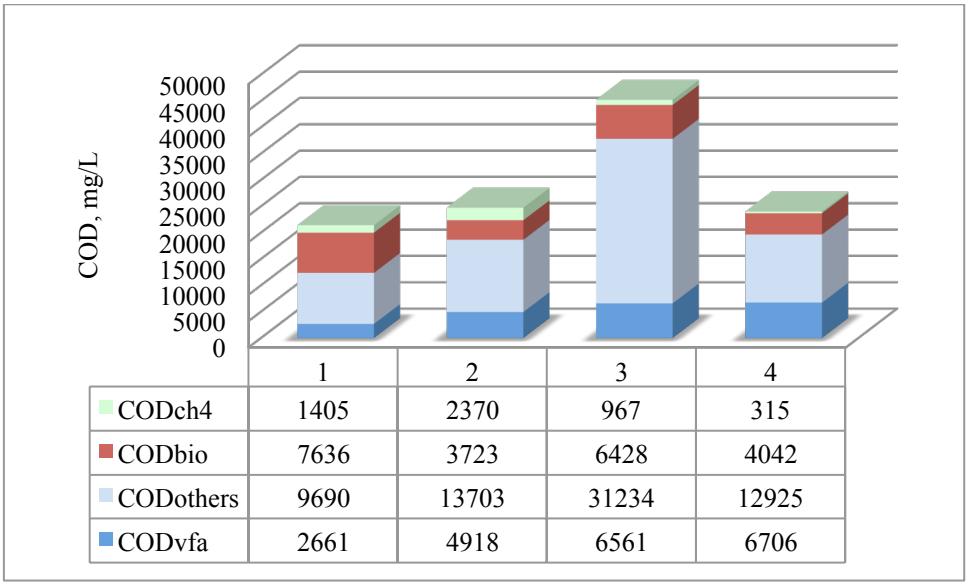


Fig. 6.24 – The relation between the COD_{in} and COD_{sum2} (3rd step)

As it can be seen in Figure 6.24 the values of COD_{in} and COD_{sum2} are almost balanced in opposite to what happened in step 2 of the mass balance. These findings show that the way to measure the biomass growth in this case (Step 3) is much better than in previous step (Step 2) of the mass balance. The Phases 1 and 2 reflect that COD_{sum2} shows the transformation of organic matter in the feed is higher than COD_{in}. In the case of Phase 1 this finding is due to the unattached biomass, which was washed out from the reactor and accumulated in the clarifier, contributing to a higher COD_{sum2} than it should be. In the case of Phase 2 – the probable reason for that was that a lot of biomass had been accumulating inside the reactor, which led to an increased amount being washed out, which in its turn reacted the clarifier and contributed to the increase in the COD_{sum2} value.

It is also necessary to point out that COD_{out} is lower than COD_r, which also contributed to COD_{sum2} (Step 3) to be lower than COD_{sum1} (Step 2). These results show that this way to measure the biomass growth is much better than to use the values from inside the reactor.

When during the intermediate stages there are some deviations due to the reactor performance and its conditions, during the last – 4th step – the mass balance is closed with the small uncertainty. So, it is possible to conclude that the system is mainly balanced with the constituents accounted in the calculations.



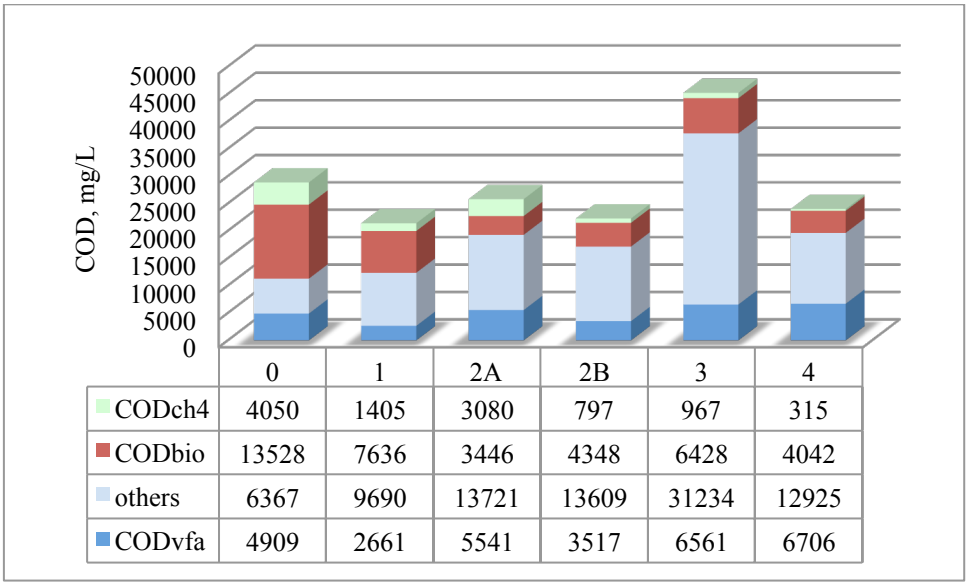
	1	2	3	4
CODch4	1405	2370	967	315
CODbio	7636	3723	6428	4042
CODothers	9690	13703	31234	12925
CODvfa	2661	4918	6561	6706

Fig. 6.25 – The data from the 4th step of mass balance scheme

The Step 4 of the mass balance is described in Figure 6.25 in a more detailed way than Step 3 (Figure 6.24). It can be seen that the higher amounts of VFAs are obtained in Phases 3 and 4, and the higher amounts of biomass are obtained in Phases 1 and 3.

Looking at Phases 3 and 4 it can be also seen that there is a lot of organic matter that is not transformed into VFAs in Phase 3. Hence, Phase 4 is the one, which presents the better performance in terms of VFAs production.

In order to better understand the MBBR reactor performance regarding all conditions studied, Phase 2 was split into two Phases (A and B – with 2 different alkalinity additions), and it was added as well as the data from the previous study (Phase 0). So, Figures 6.26 and 6.27 present the data for all Phases 0-4 and also for Phases A and B.



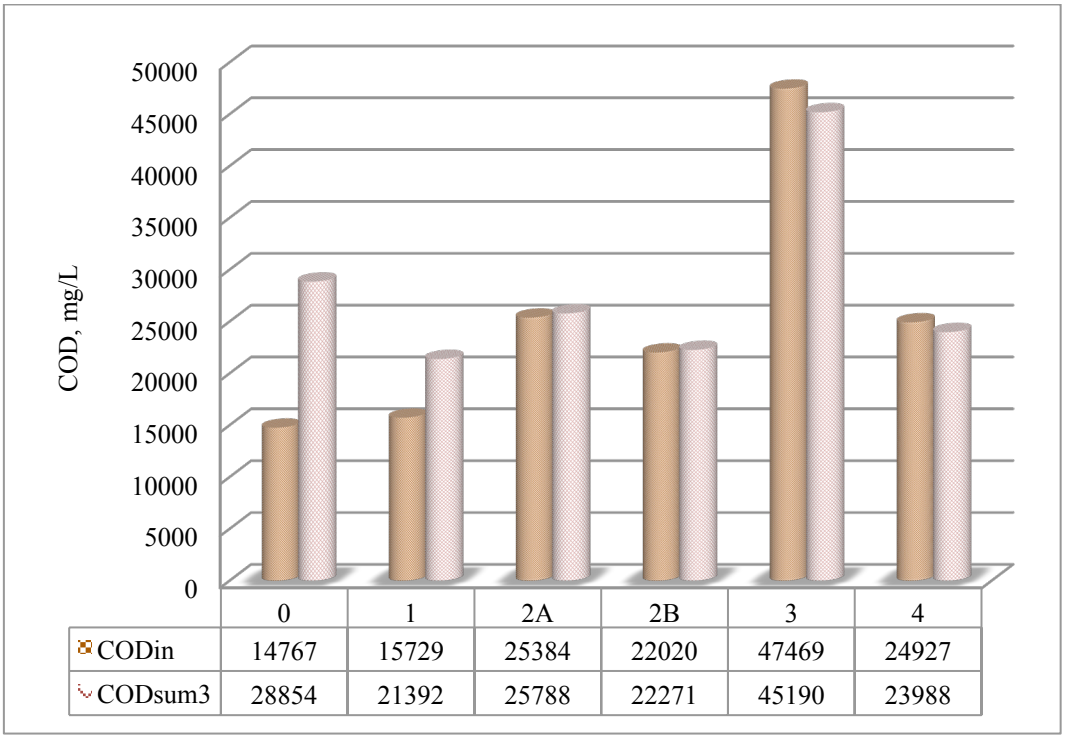
	0	1	2A	2B	3	4
CODch4	4050	1405	3080	797	967	315
CODbio	13528	7636	3446	4348	6428	4042
others	6367	9690	13721	13609	31234	12925
CODvfa	4909	2661	5541	3517	6561	6706

Fig. 6.26 – The data of 4th step of mass balance including the data from Phase 0 (previous study) and Phases A-B (split of Phase 2)

Looking at Figure 6.26 it may seem that Phase 4 represents the best results according to the high VFA yield production and low biomass production. VFA yield production during Phase 4 is on average 34% from COD_{out} (OLR = 35 gCOD/L*d). During Phase 0 (OLR = 21 gCOD/L*d) the VFA yield production is almost 44% of COD_{out}, but there is a lot of biomass in the clarifier, due to, probably, to the fact that the bacteria were not well attached to the carriers, which caused the higher wash out. The amount of methane was higher, apparently, due to the methanogenic bacteria being attached to the carriers

and protected from outside influence of the acidic environment.

At Phase 4, this did not happen because the reactor was already running for more than 100 days, which had caused the development of acidogenic bacteria in detriment of the methanogenic bacteria. Hence, the methane production was not high at Phase 4, and more VFAs were produced in comparison to Phase 0. Comparing Phase 2A and 4 for almost the same OLR (33 and 35 gCOD/L*d, respectively), it can be seen that the performance is similar, despite the higher VFA yield production at Phase 4.



	0	1	2A	2B	3	4
CODin	14767	15729	25384	22020	47469	24927
CODsum3	28854	21392	25788	22271	45190	23988

Fig. 6.27 – The relation between the COD_{in} and COD_{sum3} including the data from Phase 0 and Phases A-B (4^{th} step)

As it can be seen in Figure 6.27 the system is not balanced during Phase 0 (previous study). Apparently, it happened because the system could not reach the steady state on time within the period of experiment (the duration of experiment was around 48 days) or took too long time that influenced the average values. For all phases of study in this work, the mass balance between COD_{in} and total COD transformed are closed enough, showing that the methodology used in the steps was quite good.

To analyse the effect of the operational conditions of reactor performance regarding the

acidification of cheese-whey, for these experiments and the results from the previous study, the Table 6.12 was built.

Tab. 6.12 – Comparison results between Phase 0 (previous set of experiments) and Phases 1-4 (current experiment) [15]

Phase #	average COD_{in}, mg/L		average COD_r, mg/L	COD_r/ COD_{in} %		average COD_{out}, mg/L	COD_{out}/ COD_{in}, %	COD_{out}/ COD_r, %		average COD_{VFA}, mg/L	COD_{VFA}/ COD_{out}, %	COD_{VFA}/ COD_{in}, %
0	14767		*	*		11276	76.4	*		4909	43.5	33.2
1	15729		14943	95		12351	78.5	82.7		2661	21.5	16.9
2A	25384		28430	112		19262	75.9	67.8		5541	28.8	21.8
2B	22020		24662	112		17126	77.8	69.4		3517	20.5	16.0
3	47469		*	*		37795	79.6	*		6561	17.4	13.8
4	24927		22684	91		19631	78.8	86.5		6706	34.2	26.9
*		-	no data due to the difficulty in collecting samples from the MBBR reactor									

Looking at Table 6.12 it can be seen that the results of VFA yield production (44% and 33%, respectively, of COD_{out} and COD_{in}) at Phase 0 (OLR = 21 gCOD/L*d) are better than ones obtained at Phase 4 (OLR = 35 gCOD/L*d), which are 34% and 27%, respectively. However, the total VFA is on 27% higher in Phase 4 (6.7 gCOD/L*d) than in Phase 0 (4.9 gCOD/L*d). It can be seen that the COD in the reactor is about 91-95% of COD_{in}, except for Phase 2 where the reactor was becoming overloaded. COD_{out} was about 76-80% of COD_{in}, which is similar to any anaerobic acidification process.

7 Conclusion

In conclusion it is important to say that the experiment consisted of Phases 1-4 according to the main objective of study and Phases A-B – according to the specific one.

Among all 4 phases of experiment the 4th one with an OLR of 35 gCOD/L*d and an alkalinity of 3.0 g$NaHCO_3$/L*d presented the best results. Acidogenic fermentation of cheese-whey in a continuous fermentation experiment during this phase resulted in quite significant ratios of VFA yield to COD_{out} and COD_{in}, which are 34% and 27.5%, respectively. The highest amounts of acids of main concern – accounting for 65% of the total VFA yield production – Acetic, Propionic and i-Butyric ones were also observed during this phase – Phase 4.

The secondary objective set consisted of Phases A-B demonstrated an increase of inlet alkalinity from 3.6 to 4.1 g$CaCO_3$/L does not improve the total production of acids as well as the acids of concern for this study. The result showed that, despite the relatively small increase on the Propionic acid amount, the global amount of VFAs decreased dramatically to 50% as well as the amount of Acetic acid which dropped down to approximately 90%.

The composition of individual VFAs produced was significantly affected by the OLR applied to the MBBR reactor. The OLR also influenced the methane production. Methane production reduced from a maximum of 3.0 L/d at an organic load of 33 gCOD/L*d to zero at an OLR of 66 gCOD/L*d. It is important to mention that after lowering the organic load from 66 to 35 gCOD/L*d, methane was not being produced, apparently, due to the specific bacteria present at that time inside the reactor. So, at this load the acidogenic biomass started producing more VFAs, and methanogenic microorganisms were suppressed, although the OLR was not that high.

There were other parameters, which influenced the acidogenic fermentation process, such as the speed of mixing device also such factors as speed of mixing, the level of biomass attachment to the carrier elements and an entrapment inside the reactor. It figured out that the most appropriate speed of mixing is moderate and about 130 rpm. This parameter occurred to be in a tight connection to the biomass

attachment/entrapment process of retaining biomass inside the reactor. The speed increase or decrease led to either wash out of biomass into the clarifier or accumulation inside the MBBR reactor, respectively, which caused the MBBR overload in both cases.

Referring to previous study – Phase 0 – the results obtained at an OLR of 21 gCOD/L*d and alkalinity of 3.0 g$NaHCO_3$/L*d (the conditions applied are similar to the ones of Phase 1) look better than the data obtained at the best phase of this experimental set-up (Phase 4). The highest amounts of acids of interest are allocated to Phase 0: 70% of total VFA, at OLR = 21 gCOD/L*d, against Phase 4 with 65% at an OLR = 35 gCOD/L*d. In addition, ratios of VFA yield to COD_{out} and COD_{in} are 43.5% and 33.0% for Phase 0 against 34% and 27.5% for Phase 4, respectively.

These findings are important, because it seems that acidification of cheese-whey is maximised for low loading rates.

The results of the study showed that cheese-whey can be used as a fermentation substrate for the production of VFAs which is in its turn a source for biopolymer production. Moreover, the VFA production by cheese-whey anaerobic fermentation addresses the costly problem of the disposal of cheese-whey.

8 Suggestions for future work

Generally, according to the data obtained in the study the results seem to be quite optimistic. However, for future work it is necessary to change the operational parameters such as OLR, HRT and pH, in order to optimise the anaerobic acidification process with respect to the acidification degree and also to the important acids for polymer production.

The OLRs used in the experiments showed a tendency of obtaining better results of VFA yield production at smaller organic rates applied (20 to 35 gCOD/L*d). Hence, it seems that the OLR lower than 20 gCOD/L should be applied.

To avoid methane production at low organic loads other measures could be applied: the substitution of the MBBR reactor, the promotion of sludge circulation, the application of lower pH.

In addition, because the hydraulic regime influences the reactor performance it could be applied intermittent hydraulic regime instead of continuous one used in this study in order to allow acidogenic bacteria to handle higher loads.

Finally, the substitution of the substrate can be an option. If for cheese-whey at OLR = 66 gCOD/L*d and HRT = 12 h the VFAs production dropped down and its composition changed in a bad way considering the interest of this study, then, probably, for any other substrate with different physical, chemical and biological characteristics it may be perfect conditions.

9 References

1. Dr. Sang Yup Lee, M.Eng. Si Jae Park, M.Eng. Jong Pil Park, M.Eng. Young Lee, M.Eng. Seung Hwan Lee (2003), «Economic aspects of biopolymer production», Biopolymers, General aspects and special applications, Vol. 10, No. 18, p. 307-315;
2. http://www.globalchange-discussion.org (acsessed on the 10th of June, 2011);
3. Emrah Alkaya, Goeksel N. Demirer (2010), «Anaerobic acidification of sugar beet processing wastes: Effect of operational parameters», Biomass and Bioenergy, Vol. 35, Issue 1, p. 32-39;
4. Jeffery S. Weiss, Marcos Alvarez, Chi-Chung Tang, Robert W. Horvath, James F. Stahl (2005), «Evaluation of moving bed biofilm reactor technology for enchancing Nitrogen removal in a stabilisation pond treatment plant» WEFTEC brochure, p. 2086-2102;
5. electronic source: http://www.anoxkaldnes.com (acsessed on the 10th of January, 2011);
6. L.S. Serafim, P.C. Lemos, M.G.E. Albuquerque, M.A.M. Reis (2008), «Strategies for PHA production by mixed cultures and renewable waste metarials» Appl Microbiol Biotechnol, Vol. 81 No. 4, p. 615-628;
7. Salmiati, Z. Ujang, M.R. Salim, M.F. Md Din, M.A. Ahmad (2007), «Intracellular biopolymer productions using mixed microbial cultures from fermented POME», IWA Publishing, Water Science & Technology, Vol. 56 No. 8, p. 179-185;
8. M.G.E. Albuquerque, M. Eiroa, C. Torres, B.R. Nunes, M.A.M. Reis (2007), «Strategies for the development of a side stream process for polyhydroxyalkanoate (PHA) production from sugar cane molasses», Journal of Biotechnology, Vol. 130, Issue 4, p. 411-421;
9. Khanafari, A. Akhavan Sepahei (2007), «Alginate biopolymer biopolymer production by Azotobacter chroococcum from whey degradation», CEERS, International Journal of Environmental Science and Technology, Vol. 4, No. 4, p. 427-432;
10. R. Borja, E. Sanchez, B. Rincon, F. Raposo, M.A. Martin, A. Martin (2005), «Study and optimisation of the anaerobic acidogenic fermentation of two-phase olive pomace», Process Biochemistry, Issue 1, p. 281-291;

11. © Copyright 1999 by American Public Health Association, American Water Works Association, Water Environment Federation, Standard Methods for the Examination of Water and Wastewater (5220 D);
12. © Copyright 1999 by American Public Health Association, American Water Works Association, Water Environment Federation, Standard Methods for the Examination of Water and Wastewater (2540 D, 2540 E, 2540 G);
13. © Copyright 1999 by American Public Health Association, American Water Works Association, Water Environment Federation, Standard Methods for the Examination of Water and Wastewater (2320);
14. Bengtsson S., (2008), «Acidogenic fermentation of industrial wastewaters: Effects of chemostat retention time and pH on volatile fatty acids production», Biochemical Engineering Journal, Vol. 40, Issue 3, p. 492-499;
15. Lanko I., (2010), «Anaerobic pre-acidification of organic wastewaters for biopolymers production», Aveiro: University of Aveiro.

10 Appendix

#1.1 – The VFA concentrations obtained during Phases 1-2B

Code #	Time (d)	Peak area							Concentration (mg/L)						
		C2	C3	C4	C4	C5	C5	C6	C2	C3	C4	C4	C5	C5	C6
		Ac	Pr	i-Bu	n-Bu	i-Va	n-Va	n-Ca	Ac	Pr	i-Bu	n-Bu	i-Va	n-Va	n-Ca
		M							453	926	1054	1121	1337	1262	1241
		B							80059	59328	105437	79591	185078	12956	1052
I1		164835	431585	306545	2216621	376647	1127666	132355	187	402	191	1906	143	883	106
I2	**8**	208585	372884	137653	2810042	430989	1361765	683423	284	339	31	2435	184	1069	550
I3	**11**	1137892	327725	131352	3028970	371216	1036127	859985	2337	290	25	2631	139	811	692
I5	**18**	245483	377028	80521	1180346	401841	487231	222877	365	343	-	982	162	376	179
I6	**22**	124974	412662	77631	1345019	301774	590600	228015	99	382	-	1129	87	458	183
I7	**24**	197406	628416	92655	2398018	400208	1279748	573109	259	615	-	2068	161	1004	461
I7M	**24**	247161	33210	25205	74265	2626313	317015	730852	369	-	-	-	1825	241	588
I8	**26**	85179	31449	3169696	341533	928174	197628	330358	11	-	2907	234	556	146	265
I9	**32**	793910	910673	2421719	268178	916114	59769	222961	1577	919	2197	168	547	37	179
I9M	**32**	199594	781965	75780	1233143	285988	588770	106277	264	780	-	1029	75	456	85
I10	**36**	1747213	1328074	1298179	210723	541696	301581	115643	3683	1370	1131	117	267	229	92
I11	**39**	1851795	1220545	3947262	224098	730751	297167	194516	3914	1254	3644	129	408	225	156
I12	**43**	97979	649018	24790	55769	6340279	221956	241825	40	637	-	-	4602	166	194
I12M	**43**	776838	978008	81582	7839099	341570	1015717	558867	1539	992	-	6921	117	794	449
I13	**46**	821085	816464	13555	5386979	28930	766562	504568	1637	818	-	4734	-	597	406
I14	**50**	2356427	842730	136503	3179986	331500	1378820	567063	5028	846	29	2765	109	1082	456
I14M	**50**	3152580	1153121	178983	3925321	363164	1686076	835400	6787	1181	70	3430	133	1325	672

I15	**53**	2557824	851549	144059	3401769	370371	1543435	990901	5473	856	37	2963	139	1212	798
I16	**57**	1332504	837141	239702	3382477	565015	1617024	1461466	2766	840	127	2946	284	1271	1177
I17	**60**	1198389	858235	235980	3470825	346554	1456315	2517013	2470	863	124	3025	121	1143	2027
I18	**64**	95589	155076	27367	658315	137647	1773516	624348	34	103	-	516	-	1395	502

#1.1 – The VFA concentrations obtained during Phases 1-2B (continuation)

Code #	**Time (d)**	**Concentration as COD (mg COD/L)**							**Total COD (mg COD/L)**	**Y (g VFA /g COD)**
		C2	**C3**	**C4**	**C4**	**C5**	**C5**	**C6**		
		Ac	**Pr**	**i-Bu**	**n-Bu**	**i-Va**	**n-Va**	**n-Ca**		
		thOD								
		1.067	**1.514**	**1.818**	**1.818**	**2.039**	**2.039**	**2.207**		
I1	-	-	-	-	-	-	-	-	-	-
I2	**8**	266	224	17	1340	90	524	249	**2709**	**0.24**
I3	**11**	2190	191	14	1447	68	398	314	**4621**	**0.39**
I5	**18**	342	227	-	540	79	184	81	**1441**	**0.11**
I6	**22**	93	252	-	621	43	224	83	**1301**	**0.10**
I7	**24**	243	406	-	1137	79	492	209	**2560**	**0.20**
I7M	**24**	346	-	-	-	895	118	266	**1563**	**0.13**
I8	**26**	11	-	1599	129	273	72	120	**2183**	**0.18**
I9	**32**	1478	607	1209	93	268	18	81	**3753**	**0.36**
I9M	**32**	247	515	-	566	37	224	38	**1613**	**0.16**
I10	**36**	3451	905	622	64	131	112	42	**5328**	**0.54**
I11	**39**	3668	828	2005	71	200	110	71	**6953**	**0.43**
I12	**43**	37	421	-	-	2257	81	88	**2830**	**0.14**

I12M	**43**	1442	655	-	3807	57	390	204	**6543**	**0.29**
I13	**46**	1534	540	-	2604	-	293	184	**5049**	**0.26**
I14	**50**	4712	559	16	1521	54	531	207	**7600**	**0.43**
I14M	**50**	6361	780	38	1887	65	650	305	**10086**	**0.51**
I15	**53**	5129	565	20	1630	68	595	361	**8368**	**0.41**
I16	**57**	2593	555	70	1620	139	623	533	**6134**	**0.28**
I17	**60**	2315	570	68	1664	59	561	919	**6155**	**0.30**
I18	**64**	32	68	-	284	-	684	228	**1238**	**0.06**

#1.2 – The VFA concentrations obtained during Phases 2B-4

Code #	Time (d)	**Peak area**							**Concentration (mg/L)**						
		C2	**C3**	**C4**	**C4**	**C5**	**C5**	**C6**	**C2**	**C3**	**C4**	**C4**	**C5**	**C5**	**C6**
		Ac	**Pr**	**i-Bu**	**n-Bu**	**i-Va**	**n-Va**	**n-Ca**	**Ac**	**Pr**	**i-Bu**	**n-Bu**	**i-Va**	**n-Va**	**n-Ca**
		M							**359**	**606**	**1270**	**1170**	**1392**	**1386**	**610**
		B							**54937**	**81069**	**28959**	**141670**	**177048**	**19320**	**124**
I19	67	173369	923299	148155	2241233	302276	1162728	95361	330	1390	94	1795	90	825	156
I20	71	101044	976448	155663	2870497	326595	1429744	104712	128	1477	100	2333	107	1018	171
I20M	71	304833	1117229	269875	4163978	4163978	1700869	97940	696	1709	190	3438	2863	1214	160
I21	74	504036	620056	149580	2390429	266649	802914	14745	1251	889	95	1922	64	566	24
I22	78	160231	742902	111543	6688378	358561	2296852	66136	293	1092	65	5596	130	1644	108
I23	81	280885	1282950	158909	6908805	510140	2724594	73098	629	1983	102	5785	239	1952	120
I24	84	538405	1145848	163699	4786671	510375	965280	63170	1347	1757	106	3971	239	683	103
I25	88	275356	1491726	190550	7838794	426953	2653791	80857	614	2327	127	6580	179	1901	132
I26	92	238999	1798009	198964	9082098	440510	4092092	138433	513	2833	134	7643	189	2939	227

I27	94	159111	100003	109284	6468731	285360	1573956	22147	290	31	63	5409	78	1122	36
I28	99	162449	753577	67033	7270734	285482	3043078	82885	299	1110	30	6094	78	2182	136
I29	102	202865	1420944	0	9032048	423739	4151111	135732	412	2211	-	7600	177	2982	222
I30	106	859317	710952	78267	6912886	192042	199087	3660978	2240	1039	39	5788	11	130	5998
I32	113	603301	686666	18192	4374566	657050	28660	925718	4910	1129	13	2050	185	8	1768
I32M	113	993896	1233469	21136	5791724	275517	111274	1168662	3856	2121	1	4464	-	72	2835
I33	116	599944	492430	22605	2672511	151182	81773	404138	1518	679	-	2163	-	45	662
I34	120	1785479	1023796	34605	3849374	580060	65931	1253786	4820	1555	4	3170	289	34	2054
I35	123	1817899	765208	45316	2539952	435292	30827	1079219	4910	1129	13	2050	185	8	1768
I36	127	1439294	1366618	30466	5364159	150813	119385	1730445	3856	2121	1	4464	-	72	2835
I36M	127	598933	680848	19110	3771787	144020	62979	1113528	1515	990	-	3103	-	32	1824
I37	130	1276659	704146	42512	3350680	154745	62282	856909	3403	1028	11	2743	-	31	1404
I37M	130	1155389	655955	95594	2391966	142457	180046	118817	3065	948	52	1924	-	116	194
I38	134	923791	749746	22488	3299471	178576	1054627	538652	2420	1103	-	2699	1	747	882
I38M	134	986236	543779	27146	4298147	239701	1194325	909141	2594	763	-	3553	45	848	1489
I39	137	1701938	458629	90618	3750538	217332	732861	477151	4587	623	49	3085	29	515	782
I39M	137	2019359	544308	54177	4240753	278993	758377	490739	5471	764	20	3504	73	533	804

#1.2 – The VFA concentrations obtained during Phases 2B-4 (continuation)

Code #	Time (d)	Concentration as COD (mg COD/L)							Total COD (mg COD/L)	Y (g VFA /g COD)
		C2	C3	C4	C4	C5	C5	C6		
		Ac	Pr	i-Bu	n-Bu	i-Va	n-Va	n-Ca		
		thOD								
		1.067	1.514	1.818	1.818	2.039	2.039	2.207		
I19	67	309	918	52	987	44	405	71	**2785**	**0.17**
I20	71	120	976	55	1283	53	499	78	**3064**	**0.20**
I20M	71	652	1129	104	1891	1404	595	73	**5849**	**0.29**
I21	74	1172	587	52	1057	32	277	11	**3189**	**0.16**
I22	78	275	721	36	3078	64	806	49	**5029**	**0.14**
I23	81	590	1310	56	3182	117	958	54	**6267**	**0.18**
I24	84	1262	1160	58	2184	117	335	47	**5164**	**0.16**
I25	88	575	1537	70	3619	88	932	60	**6882**	**0.17**
I26	92	480	1871	74	4204	93	1442	103	**8266**	**0.22**
I27	94	272	21	35	2975	38	550	16	**3907**	**0.09**
I28	99	281	733	16	3352	38	1070	61	**5552**	**0.15**
I29	102	386	1460	-	4180	87	1462	101	**7664**	**0.14**
I30	106	2100	686	21	3184	5	64	2718	**8778**	**0.14**
I32	113	4602	746	7	1128	91	4	801	**7378**	**0.23**
I32M	113	3614	1401	1	2456	-	35	1285	**8781**	**0.34**
I33	116	1423	448	-	1190	-	22	300	**3371**	**0.15**
I34	120	4517	1027	2	1743	142	16	931	**8379**	**0.40**

I35	123	4602	746	7	1128	91	4	801	**7378**	**0.40**
I36	127	3614	1401	1	2456	-	35	1285	**8781**	**0.45**
I36M	127	1420	654	-	1707	-	15	827	**4607**	**0.27**
I37	130	3189	679	6	1509	-	15	636	**6026**	**0.40**
I37M	130	2872	626	29	1058	-	57	88	**4719**	**0.29**
I38	134	2268	729	-	1485	1	366	400	**5245**	**0.29**
I38M	134	2431	504	-	1954	22	416	675	**6002**	**0.33**
I39	137	4299	411	27	1697	14	253	354	**7055**	**0.38**
I39M	137	5128	505	11	1927	36	262	364	**8232**	**0.49**
	-	the days when the Phases start and finish								
	-	the VFA concentrations data obtained from samples taken from the MBBR reactor								

#2.1 – The main parameters of the system such as OLR, alk_{in}, alk_{out}, pH, COD removal rate and others during Phases 1-4

Date	**Sampling DAY**	**Time period (d)**	**OLR (gCOD/L*d)**	**alk added**		**Q (L/d)**		**S in (mgCOD/L)**				**S out (mgCOD/L)**					**Rem COD (%)**
				m NaHCO (g)	**alk_{in} (mgCaO$_3$/L)**	**Q_{real} (L/d)**	**f.d.**	**readings**			**COD in**	**f.d.**	**readings**			**COD out**	
17.01.11	0	0	20.47	3.1	3631	3.55	0.03	435	447	436	**14644**	-	-	-	-	-	
21.01.11	1	4	21.07	3.1	3631	3.55	0.03	451	459	447	**15078**	0.03	361	381	366	**12311**	**15.9%**
25.01.11	2	4	21.91	3.0	3627	3.55	0.03	494	448	469	**15678**	0.03	332	351	348	**11456**	**24.0%**
28.01.11	3	3	22.80	3.0	3629	3.55	0.03	514	473	481	**16311**	0.03	315	361	389	**11833**	**24.5%**
02.02.11	4	5	20.87	3.1	3631	3.55	0.03	458	440	446	**14933**	-	-	-	-	-	**-**
04.02.11	5	2	21.68	3.1	3631	3.55	0.03	473	465	458	**15511**	0.03	385	416	412	**13478**	**9.7%**

08.02.11	6	4	22.92	3.0	3627	3.55	0.03	496	488	492	16400	0.03	405	396	386	13189	15.0%
11.02.11	7	3	21.77	3.1	3631	3.55	0.03	474	455	473	15578	0.03	412	375	378	12944	21.1%
11.02.11	7	-	31.16	3.1	3631	3.55	0.03	474	455	473	15578	0.03	349	363	372	12044	26.6%
15.02.11	8	4	23.71	3.0	3627	3.55	0.03	466	480	581	16967	0.03	361	395	332	12089	22.4%
18.02.11	9	3	22.84	3.1	3631	3.55	0.03	475	477	519	16344	0.03	298	333	304	10389	38.8%
18.02.11	9	-	22.84	3.1	3631	3.55	0.03	475	477	519	16344	0.03	301	284	296	9789	42.3%
22.02.11	10	4	34.83	3.0	3627	3.55	0.03	782	717	744	24922	0.03	284	323	277	9822	39.9%
22.02.11	10	-	34.83	3.0	3627	3.55	0.03	782	717	744	24922	0.03	281	281	297	9544	41.6%
25.02.11	11	3	32.52	3.1	3631	3.55	0.03	711	691	692	23267	0.03	479	486	475	16000	35.8%
01.03.11	12	4	32.78	3.0	3627	3.55	0.03	676	696	739	23456	0.03	628	601	567	19956	14.2%
01.03.11	12	-	32.78	3.0	3627	3.55	0.03	676	696	739	23456	0.03	658	680	681	22433	3.6%
04.03.11	13	3	33.45	3.1	3631	3.55	0.03	718	712	724	23933	0.03	615	578	557	19444	17.1%
08.03.11	14	4	40.10	3.0	3627	3.55	0.03	853	854	875	28689	0.03	515	535	529	17544	26.7%
08.03.11	14	-	40.10	3.0	3627	3.55	0.03	853	854	875	28689	0.03	562	603	631	19956	16.6%
11.03.11	15	3	36.70	3.1	3631	3.55	0.03	770	813	780	26256	0.03	633	630	590	20589	28.2%
15.03.11	16	4	35.39	3.0	3627	3.55	0.03	748	760	771	25322	0.03	659	639	650	21644	17.6%
15.03.11	16	-	-	3.0	3627	3.55	0.03	-	-	-	-	0.03	-	-	-		-
18.03.11	17	3	37.16	3.1	3631	3.55	0.03	747	849	797	26589	0.03	619	588	657	20711	18.2%
22.03.11	18	4	30.93	3.0	4127	3.55	0.03	622	668	702	22133	0.03	430	447	468	14944	43.8%
22.03.11	18	-	30.93	3.0	4127	3.55	0.03	622	668	702	22133	0.03	408	396	398	13356	49.8%
25.03.11	19	3	30.83	3.1	4167	3.55	0.03	647	670	668	22056	0.03	492	474	467	15922	28.1%
29.03.11	20	4	30.41	3.0	4127	3.55	0.03	668	649	641	21756	0.03	444	471	488	15589	29.3%
29.03.11	20	-	30.41	3.0	4127	3.55	0.03	668	649	641	21756	0.03	634	620	547	20011	9.3%
01.04.11	21	3	58.77	3.1	3631	3.55	0.02	846	851	826	42050	0.02	405	396	391	19867	8.7%

05.04.11	22	4	59.91	3.0	3627	3.55	0.02	850	860	862	42867	0.03	1046	1071	1041	35089	16.6%
08.04.11	23	3	59.14	3.1	3631	3.55	0.02	871	842	826	42317	0.03	1036	1056	1040	34800	18.8%
12.04.11	24	4	64.80	3.0	3627	3.55	0.02	977	839	966	46367	0.02	623	656	678	32617	22.9%
15.04.11	25	3	68.09	3.1	3631	3.55	0.02	989	946	988	48717	0.02	791	820	796	40117	13.5%
19.04.11	26	4	73.98	3.1	3631	3.55	0.01	496	549	543	52933	0.02	739	775	722	37267	23.5%
21.04.11	27	2	77.34	3.0	3629	3.55	0.01	608	540	512	55333	0.01	451	449	430	44333	16.2%
26.04.11	28	5	68.72	3.0	3627	3.55	0.01	492	487	496	49167	0.01	369	379	381	37633	32.0%
29.04.11	29	3	71.19	3.1	3631	3.55	0.01	501	506	521	50933	0.01	574	569	539	56067	-14.0%
03.05.11	30	4	74.49	3.0	3627	3.55	0.01	541	524	534	53300	0.01	632	616	645	63100	-23.9%
06.05.11	31	3	74.45	3.1	3631	3.55	0.01	542	530	526	53267	0.01	651	623	633	63567	-19.3%
10.05.11	32	4	37.27	3.0	3627	3.55	0.01	244	277	279	26667	0.01	303	334	325	32067	34.8%
10.05.11	32	-	37.27	3.0	3627	3.55	0.01	244	277	279	26667	0.01	231	256	293	26000	47.1%
13.05.11	33	3	39.15	3.1	3631	3.55	0.03	851	813	857	28011	0.03	633	620	752	22278	16.5%
17.05.11	34	4	33.54	3.0	3627	3.55	0.03	752	715	693	24000	0.03	631	658	582	20789	25.8%
20.05.11	35	3	37.29	3.1	3631	3.55	0.03	814	776	811	26678	0.03	539	559	570	18533	22.8%
24.05.11	36	4	30.89	3.0	3627	3.55	0.03	676	607	706	22100	0.03	622	600	542	19600	26.5%
24.05.11	36	-	30.89	3.0	3627	3.55	0.03	676	607	706	22100	0.03	521	543	497	17344	35.0%
27.05.11	37	3	33.33	3.1	3631	3.55	0.03	723	710	713	23844	0.03	482	437	447	15178	31.3%
27.05.11	37	-	33.33	3.1	3631	3.55	0.03	723	710	713	23844	0.03	484	493	485	16244	26.5%
31.05.11	38	4	32.41	3.0	3627	3.55	0.03	679	702	706	23189	0.03	537	549	550	18178	23.8%
31.05.11	38	-	32.41	3.0	3627	3.55	0.03	679	702	706	23189	0.03	507	557	549	17922	24.8%
03.06.11	39	3	-	-	-	3.55	0.03	-	-	-	#DIV/0!	0.03	568	562	541	18567	19.9%
03.06.11	39	-	-	-	-	3.55	0.03	-	-	-	#DIV/0!	0.03	486	497	523	16733	27.8%

Notes:

	-	the data which correspond to the Phase 1
	-	the data which correspond to the Phase 2A
	-	the data which correspond to the Phase 2B
	-	the data which correspond to the Phase 3
	-	the data which correspond to the Phase 4
	-	the COD_{out} data obtained from samples taken from the MBBR reactor

#2.1 – The main parameters of the system such as OLR, alk_{in}, alk_{out}, pH, COD removal rate and others during Phases 1-4 (continuation)

Date	Sampling DAY	Time period	pH	V HCl (mL)	[H2SO4] (mol/l	V (mL)	alk_{out} (mg $CaCO_3$/L)	TSS (mg/L)	VSS (mg/L)
17.01.11	0	0	-	-	-	-	-	-	-
21.01.11	1	4	4.81	6.3	0.1	50	630	-	-
25.01.11	2	4	4.93	8.75	0.1	50	875	1147	1033
28.01.11	3	3	5.08	13.5	0.1	50	1350	7253	6780
02.02.11	4	5	-	-	0.1	50	0	0	0
04.02.11	5	2	4.74	6.6	0.1	50	660	0	0
08.02.11	6	4	4.59	1.35	0.1	50	135	7007	6687
11.02.11	7	3	4.84	8.5	0.1	50	850	30987	28753
11.02.11	7	-	4.84	8.5	0.1	50	850	-	-
15.02.11	8	4	5.03	10.4	0.1	50	1040	12453	11720
18.02.11	9	3	4.88	6.9	0.1	50	690	5320	4993
18.02.11	9	-	4.88	6.9	0.1	50	690	-	-
22.02.11	10	4	4.69	4.6	0.1	50	460	1180	1053
22.02.11	10	-	4.69	4.6	0.1	50	460	-	-
25.02.11	11	3	4.40	-	0.1	50	0	1227	1153

01.03.11	12	4	4.50	-	0.1	50	0	18933	2020
01.03.11	12	-	4.50	-	0.1	50	0	-	-
04.03.11	13	3	4.86	9.25	0.1	50	925	1627	1473
08.03.11	14	4	4.61	4.05	0.1	50	405	1640	1473
08.03.11	14	-	4.61	4.05	0.1	50	405	-	-
11.03.11	15	3	4.52	1.45	0.1	50	145	1713	1580
15.03.11	16	4	4.45	-	0.1	50	0	4160	3980
15.03.11	16	-	4.45	-	0.1	50	0	-	-
18.03.11	17	3	4.50	-	0.1	50	0	4000	3713
22.03.11	18	4	4.83	8.5	0.1	50	850	3333	3140
22.03.11	18	-	4.83	8.5	0.1	50	850	-	-
25.03.11	19	3	4.82	7.15	0.1	50	715	3480	3307
29.03.11	20	4	4.77	7.7	0.1	50	770	3720	3540
29.03.11	20	-	4.77	7.7	0.1	50	770	-	-
01.04.11	21	3	4.90	10.25	0.1	50	1025	1753	1553
05.04.11	22	4	4.55	1.7	0.1	50	170	3953	3760
08.04.11	23	3	4.80	10.6	0.1	50	1060	3713	3393
12.04.11	24	4	4.72	9.55	0.1	50	955	3060	2567
15.04.11	25	3	4.35	-	0.1	50	0	7480	6880
19.04.11	26	4	4.58	2.55	0.1	50	255	6380	5867
21.04.11	27	2	4.85	10.57	0.1	50	1057	3040	2793
26.04.11	28	5	4.44	-	0.1	50	0	22833	21040
29.04.11	29	3	4.29	-	0.1	50	0	17533	16453
03.05.11	30	4	4.35	-	0.1	50	0	24853	23213

06.05.11	31	3	-	-	0.1	50	0	0	0
10.05.11	32	4	4.78	6.35	0.1	50	635	2167	2080
10.05.11	32	-	4.70	5.8	0.1	50	-	-	-
13.05.11	33	3	4.17	-	0.1	50	0	26153	24740
17.05.11	34	4	4.72	5.75	0.1	50	575	18180	17327
20.05.11	35	3	4.71	5.45	0.1	50	545	7360	7060
24.05.11	36	4	4.66	4.65	0.1	50	465	18933	17687
24.05.11	36	-	5.07	11.15	0.1	50	-	-	-
27.05.11	37	3	4.86	8.7	0.1	50	870	3647	3487
27.05.11	37	-	5.05	11.1	0.1	50	-	-	-
31.05.11	38	4	4.62	2.55	0.1	50	255	4327	4120
31.05.11	38	-	4.83	8.65	0.1	50	865	-	-
03.06.11	39	3	4.56	1.15	0.1	50	115	1900	1700
03.06.11	39	-	4.57	1.65	0.1	50	165	-	-

Notes:

	-	the COD_{out} data obtained from samples taken from the MBBR reactor
	-	the days in the end of Phase 3, when the complete overload of the MBBR reactor happened with the consequent results

#3.1 – Biogas monitoring record

	Absolute areas				Normal area (%)			Molar const (%			Gas count (L)	V (L/d)	V_{CH4} (L/d)	$\%CO_2+\%CH_4$	COD of CH_4 (mg/L*d)
Run day	interf	CH_4	CO_2	TOTAL	interf	CH_4	CO_2	interf	CH_4	CO_2					
3	-	-	-	-	-	-	-	-	-	-	23692.11	-	-	-	-
4	1294.3025	417.3550	3490.7605	3908.1155	33.1	10.7	89.3	-3	13	89	23692.60	0.5	0.1	102.9	165.07
5	-	-	-	-	-	-	-	-	17	-	23695.94	1.9	0.3	17.0	807.5
6	-	-	-	-	-	-	-	-	20	-	23699.28	3.3	0.7	20.0	1670
7	-	-	-	-	-	-	-	-	23	-	23702.62	3.4	0.8	23.0	1955
8	377.2950	1183.7120	3823.2405	5006.9525	7.5	23.6	76.4	-2	26	76	23705.95	4.8	1.3	102.2	3126.76
9	569.0640	1083.2305	3329.3950	4412.6255	12.9	24.5	75.5	-2	27	75	23714.10	8.1	2.2	102.2	5488.35
10	365.2040	1352.0100	3556.0470	4908.0570	7.4	27.5	72.5	-2	30	72	23720.25	6.2	1.8	102.0	4588.96
11	307.9485	1199.1560	3144.4205	4343.5765	7.1	27.6	72.4	-2	30	72	23726.10	5.8	1.7	102.0	4373.74
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	pump calibration			-	-	-	-	-	-	-	-	-	-	-	-
15				-	-	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	-	-	23726.20	-	-	-	-
17	451.2795	339.2865	4247.6080	4586.8945	9.8	7.4	92.6	-3	10	93	23726.30	0.1	0.0	103.0	25.72
18	3285.9050	0.0000	2085.5275	2085.5275	157.6	0.0	100.0	-3	3	100	23727.10	0.8	0.0	103.4	62.2
19	-	-	-	-	-	-	-	-	5	-	-	0.4	0.0	5.0	50
20	-	-	-	-	-	-	-	-	6	-	-	0.4	0.0	6.0	60
21	779.4360	157.2705	3012.2770	3169.5475	24.6	5.0	95.0	-3	8	95	23728.40	0.4	0.0	103.1	85.87
22	371.4615	445.4710	4496.7105	4942.1815	7.5	9.0	91.0	-3	12	91	23730.45	2.0	0.2	102.9	607.76
23	266.2780	226.9505	4150.8510	4644.0795	5.7	4.9	89.4	3	8	89	23732.95	2.5	0.2	97.3	490.83

24	296.2890	254.1310	4353.4510	4903.8710	6.0	5.2	88.8	3	8	89	23735.15	2.2	0.2	97.0	447.69
25	400.1010	305.5480	4274.0300	4979.6790	8.0	6.1	85.8	5	9	86	23735.35	0.2	0.0	94.9	45.33
26	-	-	-	-	-	-	-	-	9	-	-	1.1	0.1	9.0	247.5
27	-	-	-	-	-	-	-	-	10	-	-	1.9	0.2	10.0	475
28	152.5500	308.1870	4211.5900	4672.3270	3.3	6.6	90.1	0	10	90	23741.50	2.3	0.2	99.7	546.94
29	314.5150	1232.9360	5195.9420	6743.3930	4.7	18.3	77.1	2	21	77	23744.60	3.1	0.6	97.7	1616.35
30	229.1420	403.9150	4787.1230	5420.1800	4.2	7.5	88.3	1	10	88	23745.40	0.8	0.1	98.7	206.86
31	647.9600	790.2970	3061.3480	4499.6050	14.4	17.6	68.0	12	20	68	23746.65	1.3	0.3	87.8	629.92
32	360.9445	1056.1690	3526.4205	4943.5340	7.3	21.4	71.3	5	24	71	23748.25	1.6	0.4	94.9	953.86
33	-	-	-	-	-	-	-	-	44	-	-	2.1	0.9	44.0	2310
34	-	-	-	-	-	-	-	-	44	-	-	2.4	1.1	44.0	2640
35	219.1550	2828.1925	1407.2940	4454.6415	4.9	63.5	31.6	5	65	31	23754.50	1.7	1.1	95.3	2751.11
36	266.2370	3247.5140	719.0395	4232.7905	6.3	76.7	17.0	7	78	16	23756.00	1.5	1.2	93.2	2909.14
37	98.6180	704.8750	4343.0560	5146.5490	1.9	13.7	84.4	-1	16	84	23765.30	9.3	1.5	100.8	3813.79
38	182.2240	854.0060	4233.3830	5269.6130	3.5	16.2	80.3	1	19	80	23774.61	9.3	1.8	99.1	4384.96
39	310.4180	730.9290	4195.6120	5236.9590	5.9	14.0	80.1	3	17	80	23781.65	7.0	1.2	96.7	2931.59
40	-	-	-	-	-	-	-	-	14	-	-	8.8	1.2	14.0	3080
41	-	-	-	-	-	-	-	-	14	-	-	9.2	1.3	14.0	3220
42	674.8860	218.3065	3426.2230	4319.4155	15.6	5.1	79.3	13	8	79	23809.12	9.6	0.8	87.2	1923.72
43	275.6090	229.0990	3706.6480	4211.3560	6.5	5.4	88.0	4	8	88	23819.45	10.3	0.9	96.4	2166.74
44	141.9080	214.1090	4176.6405	4532.6575	3.1	4.7	92.1	0	8	92	23829.27	9.8	0.8	100.0	1889.08
45	161.7260	488.7370	4279.7040	4930.1670	3.3	9.9	86.8	0	13	87	23835.26	6.0	0.8	99.6	1906.58
46	238.8940	1541.3450	2912.4010	4692.6400	5.1	32.8	62.1	3	35	62	23837.17	1.9	0.7	96.6	1670.79
47	-	-	-	-	-	-	-	-	50	-	-	3.1	1.6	50.0	3875

48	-	-	-	-	-	-	-	-	50	-	-	4.0	2.0	50.0	5000
49	141.5255	2985.6650	1474.7250	4601.9155	3.1	64.9	32.0	3	66	31	23848.25	3.4	2.2	97.1	5616.91
50	201.7720	3158.8810	1114.9850	4475.6380	4.5	70.6	24.9	5	72	24	23851.16	2.9	2.1	95.4	5209.95
51	163.7200	2144.5120	2729.5190	5037.7510	3.2	42.6	54.2	2	44	54	23857.19	6.0	2.7	98.0	6697.42
52	116.6580	3325.1280	1049.5620	4491.3480	2.6	74.0	23.4	3	75	22	23860.36	3.2	2.4	97.1	5941.17
53	191.6820	3490.1280	854.6550	4536.4650	4.2	76.9	18.8	5	78	18	23864.28	3.9	3.0	95.3	7622.74
54	-	-	-	-	-	-	-	-	13	-	-	5.7	0.7	13.0	1852.5
55	-	-	-	-	-	-	-	-	13	-	-	7.7	1.0	13.0	2502.5
56	256.3190	542.9310	4549.9445	5349.1945	4.8	10.1	85.1	2	13	85	23887.33	9.7	1.3	98.0	3143.13
57	1039.4905	239.0340	3318.3410	4596.8655	22.6	5.2	72.2	20	8	72	23900.24	12.9	1.1	80.1	2632.69
58	121.0090	146.3710	3866.8850	4134.2650	2.9	3.5	93.5	0	7	94	23910.59	10.3	0.7	100.2	1693.87
59	273.7170	76.0250	3558.2470	3907.9890	7.0	1.9	91.1	4	5	91	23922.01	11.4	0.6	96.1	1426.98
60	132.3275	76.8030	3598.9330	3808.0635	3.5	2.0	94.5	0	5	95	23935.72	13.7	0.7	99.7	1736.9
61	-	-	-	-	-	-	-	-	10	-	-	11.1	1.1	10.0	2775
62	-	-	-	-	-	-	-	-	10	-	-	9.3	0.9	10.0	2325
63	234.2560	421.9970	3725.3080	4381.5610	5.3	9.6	85.0	3	12	85	23962.98	6.9	0.9	97.5	2149.01
64	450.1850	512.8425	4146.8550	5109.8825	8.8	10.0	81.2	6	13	81	23965.66	2.7	0.3	93.9	861.03
65	139.3275	247.5710	4829.1610	5216.0595	2.7	4.7	92.6	0	8	93	23977.81	12.2	0.9	100.4	2343.97
66	121.3260	134.4070	3854.6395	4110.3725	3.0	3.3	93.8	0	6	94	23990.02	12.2	0.8	100.2	1918.13
67	172.0360	0.0000	3751.1150	3923.1510	4.4	0.0	95.6	1	3	96	24005.00	15.0	0.5	98.9	1164.69
68	-	-	-	-	-	-	-	-	3	-	-	14.4	0.4	3.0	1080
69	-	-	-	-	-	-	-	-	3	-	-	13.7	0.4	3.0	1027.5
70	125.3990	0.0000	3604.1410	3729.5400	3.4	0.0	96.6	0	3	97	24046.83	13.6	0.4	100.0	1057.4
71	192.6930	0.0000	3637.8335	3830.5265	5.0	0.0	95.0	2	3	95	24055.04	8.2	0.3	98.3	638.33

72	121.1760	0.0000	4152.9330	4274.1090	2.8	0.0	97.2	0	3	97	24063.03	**8.0**	**0.2**	100.5	621.22
73	158.5775	54.8335	3716.6840	3930.0950	4.0	1.4	94.6	1	4	95	24065.88	**2.9**	**0.1**	99.2	318.07
74	147.3455	0.0000	3789.8930	3937.2385	3.7	0.0	96.3	0	3	96	24067.00	**1.1**	**0.0**	99.6	87.08
75	-	-	-	-	-	-	-	-	3	-	-	**8.4**	**0.3**	3.0	630
76	-	-	-	-	-	-	-	-	3	-	-	**18.0**	**0.5**	3.0	1350
77	542.3450	0.0000	3833.3005	4375.6455	12.4	0.0	87.6	9	3	88	24116.90	**23.4**	**0.7**	90.7	1819.35
78	562.0810	0.0000	3758.2325	4320.3135	13.0	0.0	87.0	10	3	87	24132.62	**15.7**	**0.5**	90.1	1222.23
79	675.5820	0.0000	4094.1070	4769.6890	14.2	0.0	85.8	11	3	86	24141.45	**8.8**	**0.3**	88.9	686.53
80	579.3775	0.0000	3989.2770	4568.6545	12.7	0.0	87.3	10	3	87	24146.50	**5.0**	**0.2**	90.5	392.64
81	787.0580	0.0000	3367.1260	4154.1840	18.9	0.0	81.1	16	3	81	24149.45	**3.0**	**0.1**	84.1	229.36
82	-	-	-	-	-	-	-	-	4	-	-	**1.6**	**0.1**	4.0	160
83	-	-	-	-	-	-	-	-	4	-	-	**0.9**	**0.0**	4.0	90
84	885.5740	55.1730	3047.2145	3987.9615	22.2	1.4	76.4	19	4	76	24152.02	**0.6**	**0.0**	80.7	66.79
85	1636.1040	326.2235	1810.5085	3772.8360	43.4	8.6	48.0	41	12	47	24152.43	**0.4**	**0.0**	58.8	117.9
86	120.5790	0.0000	5512.5605	5633.1395	2.1	0.0	97.9	-1	3	98	24157.82	**5.4**	**0.2**	101.2	419.07
87	151.0920	0.0000	3936.3440	4087.4360	3.7	0.0	96.3	0	3	97	24174.08	**16.3**	**0.5**	99.6	1264.22
88	334.7000	0.0000	3858.3140	4193.0140	8.0	0.0	92.0	5	3	92	24192.59	**18.5**	**0.6**	95.2	1439.15
89	-	-	-	-	-	-	-	-	3	-	-	**15.2**	**0.5**	3.0	1140
90	-	-	-	-	-	-	-	-	3	-	-	**9.1**	**0.3**	3.0	682.5
91	210.6930	0.0000	2991.5475	3202.2405	6.6	0.0	93.4	3	3	94	24223.31	**6.3**	**0.2**	96.7	489.83
92	329.4255	0.0000	4129.8210	4459.2465	7.4	0.0	92.6	4	3	93	24224.78	**1.5**	**0.0**	95.9	114.29
93	170.0620	0.0000	4583.5480	4753.6100	3.6	0.0	96.4	0	3	97	24227.99	**3.2**	**0.1**	99.7	249.58
94	377.9685	0.0000	4190.5625	4568.5310	8.3	0.0	91.7	5	3	92	24228.70	**0.7**	**0.0**	94.9	55.2
95	-	-	-	-	-	-	-	-	3	-	-	**6.7**	**0.2**	3.0	502.5

96	-	-	-	-	-	-	-	-	3	-	-	**19.3**	**0.6**	3.0	1447.5
97	-	-	-	-	-	-	-	-	3	-	-	**29.5**	**0.9**	3.0	2212.5
98	-	-	-	-	-	-	-	-	3	-	-	**32.1**	**1.0**	3.0	2407.5
99	109.9180	0.0000	3720.9705	3830.8885	2.9	0.0	97.1	**0**	3	**97**	24338.77	**25.6**	**0.8**	100.5	1990.4
100	466.5920	0.0000	3555.8340	4022.4260	11.6	0.0	88.4	**8**	3	**88**	24363.21	**24.4**	**0.8**	91.6	1900.21
101	111.5630	0.0000	3641.9735	3753.5365	3.0	0.0	97.0	**0**	3	**97**	24388.63	**25.4**	**0.8**	100.4	1976.41
102	-	-	-	-	-	-	-	-	3	-	24411.19	**22.6**	**0.7**	3.0	1692
103	-	-	-	-	-	-	-	-	3	-	-	**31.1**	**0.9**	3.0	2332.5
104	-	-	-	-	-	-	-	-	3	-	-	**33.9**	**1.0**	3.0	2542.5
105	118.1800	0.0000	3598.5190	3716.6990	3.2	0.0	96.8	**0**	3	**97**	24504.56	**28.3**	**0.9**	100.1	2200.33
106	179.7555	0.0000	3636.1460	3815.9015	4.7	0.0	95.3	**1**	3	**95**	24523.41	**18.8**	**0.6**	98.6	1465.59
107	172.5100	0.0000	3579.6390	3752.1490	4.6	0.0	95.4	**1**	3	**96**	24523.41	**0.0**	**0.0**	98.7	0
108	160.5520	0.0000	3454.4470	3614.9990	4.4	0.0	95.6	**1**	3	**96**	24537.62	**14.2**	**0.4**	98.9	1104.83
109	224.1050	0.0000	3115.2955	3339.4005	6.7	0.0	93.3	**3**	3	**93**	24546.34	**8.7**	**0.3**	96.5	677.98
110	-	-	-	-	-	-	-	-	3	-	-	**0.8**	**0.0**	3.0	60
111	-	-	-	-	-	-	-	-	3	-	-	**0.7**	**0.0**	3.0	52.5
112	3147.9240	0.0000	1192.1295	4340.0535	72.5	0.0	27.5	**71**	3	**26**	24548.16	**0.6**	**0.0**	29.5	47.17
113	3859.7380	0.0000	970.5420	4830.2800	79.9	0.0	20.1	**78**	3	**19**	24548.62	**0.5**	**0.0**	21.9	35.76
114	127.9990	0.0000	4700.5730	4828.5720	2.7	0.0	97.3	**-1**	3	**98**	24549.66	**1.0**	**0.0**	100.7	80.86
115	167.7695	0.0000	3452.6450	3620.4145	4.6	0.0	95.4	**1**	3	**96**	24553.83	**4.2**	**0.1**	98.7	324.22
116	277.3130	0.0000	2957.5075	3234.8205	8.6	0.0	91.4	**5**	3	**92**	24557.41	**3.6**	**0.1**	94.6	278.34
117	-	-	-	-	-	-	-	-	3	-	-	**4.9**	**0.1**	3.0	367.5
118	-	-	-	-	-	-	-	-	3	-	-	**5.8**	**0.2**	3.0	435
119	383.5400	0.0000	3679.1795	4062.7195	9.4	0.0	90.6	**6**	3	**91**	24573.19	**5.2**	**0.2**	93.8	404.3

120	1726.0440	0.0000	2993.1330	4719.1770	36.6	0.0	63.4	34	3	63	24573.19	0.0	0.0	66.1	0
121	1195.0485	0.0000	3889.4370	5084.4855	23.5	0.0	76.5	21	3	76	24573.19	0.0	0.0	79.4	0
122	3397.6910	0.0000	1703.4090	5101.1000	66.6	0.0	33.4	65	3	32	24573.19	0.0	0.0	35.5	0
123	5206.1270	0.0000	270.9110	5477.0380	95.1	0.0	4.9	93	3	3	24573.19	0.0	0.0	6.5	0
124	-	-	-	-	-	-	-	-	3	-	24573.19	0.0	0.0	3.0	0
125	-	-	-	-	-	-	-	-	3	-	24573.19	0.0	0.0	3.0	0
126	441.2475	0.0000	3529.2740	3970.5215	11.1	0.0	88.9	8	3	89	24573.19	0.0	0.0	92.1	0
127	1236.0795	0.0000	3785.1895	5021.2690	24.6	0.0	75.4	22	3	75	24573.19	0.0	0.0	78.3	0
128	801.4830	0.0000	4674.1200	5475.6030	14.6	0.0	85.4	12	3	85	24573.19	0.0	0.0	88.5	0
129	3778.4260	0.0000	1737.9930	5516.4190	68.5	0.0	31.5	66	3	30	24573.19	0.0	0.0	33.6	0
130	5294.3650	0.0000	124.0800	5418.4450	97.7	0.0	2.3	96	3	1	24573.19	0.0	0.0	3.8	0
131	-	-	-	-	-	-	-	-	3	-	24573.19	0.0	0.0	3.0	0
132	-	-	-	-	-	-	-	-	3	-	24573.19	0.0	0.0	3.0	0
133	365.2455	0.0000	4052.2900	4417.5355	8.3	0.0	91.7	5	3	92	24573.19	0.0	0.0	95.0	0
134	898.9940	0.0000	4647.0870	5546.0810	16.2	0.0	83.8	13	3	84	24573.19	0.0	0.0	86.9	0
135	489.4380	0.0000	4943.7600	5433.1980	9.0	0.0	91.0	6	3	91	24573.19	0.0	0.0	94.2	0
136	445.0380	0.0000	5142.7430	5587.7810	8.0	0.0	92.0	5	3	92	24573.19	0.0	0.0	95.3	0
137	1990.7835	0.0000	3450.4055	5441.1890	36.6	0.0	63.4	34	3	63	24573.19	0.0	0.0	66.1	0

Note:

	-	extrapolated values on days when it was not possible to conduct the tests

#4.1 – TSS and VSS data obtained from the analyses performed over samples from the MBBR reactor (Replicate 1)

Sampling day	TSS (g/L)	VSS (g/L)	Replicate 1						COD of biomass (mg)
			net (g)	V (mL)	moven (g)	mmuffle (g)	TSS (g/L)	VSS (g/L)	
I5M	10.167	9.560	33.9523	5	34.0033	33.9556	10.2	9.54	13575.2
I7M	3.247	2.853	27.5964	5	27.6133	27.5984	3.38	2.98	4051.73
I9M	1.147	1.053	19.8079	5	19.8139	19.8087	1.2	1.04	1495.73
I10M	1.820	1.680	27.5974	5	27.6068	27.5983	1.88	1.7	2385.6
I12M	15.127	14.120	20.6717	5	20.7508	20.6781	15.82	14.54	20050.4
I14M	21.313	19.747	19.8138	5	19.9222	19.8218	21.68	20.08	28040.27
I16M	49.133	45.880	25.1186	5	25.3653	25.1346	49.34	46.14	65149.6
I18M	11.173	10.540	23.8364	5	23.8909	23.8399	10.9	10.2	14966.8
I20M	17.987	16.940	25.2077	5	25.3030	25.2145	19.06	17.7	24054.8
I32M	41.540	39.753	23.8367	5	24.0398	23.8452	40.62	38.92	56449.73
I36M	4.120	3.853	19.8168	5	19.8313	19.8176	2.9	2.74	5471.73
I37M	0.740	0.627	19.0667	5	19.0711	19.0673	0.88	0.76	889.87
I38M	9.533	9.067	19.7286	5	19.7762	19.7308	9.52	9.08	12874.67
I39M	14.333	13.167	30.0623	5	30.1311	30.0655	13.76	13.12	18696.67

#4.2 – TSS and VSS data obtained from the analyses performed over samples from the MBBR reactor (Replicate 2)

Sampling day	TSS (g/L)	VSS (g/L)	Replicate 2						COD of biomass (mg
			net (g)	V (mL)	moven (g)	mmuffle (g)	TSS (g/L)	VSS (g/L)	
I5M	10.173	9.600	31.5847	5	31.6358	31.5875	10.22	9.66	13575.2
I7M	3.173	2.787	25.6761	5	25.6919	25.6780	3.16	2.78	4051.73
I9M	1.127	1.073	20.6958	5	20.7015	20.6960	1.14	1.1	1495.73
I10M	1.780	1.653	25.1180	5	25.1268	25.1187	1.76	1.62	2385.6
I12M	14.953	14.007	20.6530	5	20.7295	20.6585	15.3	14.2	20050.4
I14M	21.320	19.740	19.8083	5	19.9168	19.8165	21.7	20.06	28040.27
I16M	48.813	45.573	25.6761	5	25.9180	25.6919	48.38	45.22	65149.6
I18M	11.307	10.753	19.8093	5	19.8658	19.8116	11.3	10.84	14966.8
I20M	17.333	16.487	20.6946	5	20.7801	20.6984	17.1	16.34	24054.8
I32M	41.860	40.000	30.0619	5	30.2698	30.0715	41.58	39.66	56449.73
I36M	4.767	4.433	18.5477	5	18.5719	18.5495	4.84	4.48	5471.73
I37M	0.660	0.560	25.6789	5	25.6821	25.6793	0.64	0.56	889.87
I38M	9.333	8.873	25.1191	5	25.1637	25.1212	8.92	8.5	12874.67
I39M	14.820	13.160	25.2012	5	25.2773	25.2118	15.22	13.1	18696.67

#4.3 – TSS and VSS data obtained from the analyses performed over samples from the MBBR reactor (Replicate 3)

Sampling day	TSS (g/L)	VSS (g/L)	Replicate 3						COD of biomass (mg
			net (g)	V (mL	moven (g)	mmuffle (g)	TSS (g/L)	VSS (g/L)	
I5M	10.167	9.560	31.096(	5	31.1464	31.0990	10.08	9.48	13575.2
I7M	3.247	2.853	30.0611	5	30.0771	30.0631	3.2	2.8	4051.73
I9M	1.147	1.053	19.8151	5	19.8206	19.8155	1.1	1.02	1495.73
I10M	1.820	1.680	21.2273	5	21.2364	21.2278	1.82	1.72	2385.6
I12M	15.127	14.120	19.0663	5	19.1376	19.0695	14.26	13.62	20050.4
I14M	21.313	19.747	23.8349	5	23.9377	23.8422	20.56	19.1	28040.27
I16M	49.133	45.880	21.1613	5	21.4097	21.1783	49.68	46.28	65149.6
I18M	11.173	10.540	23.6378	5	23.6944	23.6415	11.32	10.58	14966.8
I20M	17.987	16.940	30.0614	5	30.1504	30.0665	17.8	16.78	24054.8
I32M	41.540	39.753	19.7273	5	19.9394	19.7360	42.42	40.68	56449.73
I36M	4.120	3.853	20.6965	5	20.7196	20.6979	4.62	4.34	5471.73
I37M	0.740	0.627	23.8377	5	23.8412	23.8384	0.7	0.56	889.87
I38M	9.533	9.067	23.6382	5	23.6890	23.6409	10.16	9.62	12874.67
I39M	14.333	13.167	22.3314	5	22.4015	22.3351	14.02	13.28	18696.67

#5.1 – TSS and VSS data obtained from the analyses performed over samples from the CLAR (Replicate 1)

Sampling day	TSS (g/L)	VSS (g/L)	Replicate 1						COD of biomass (mg)
			net (g)	V (mL)	moven (g)	mmuffle (g)	TSS (g/L)	VSS (g/L)	
2	1.147	1.033	20.6524	5	20.6581	20.6530	1.14	1.02	1467.33
3	7.253	6.780	23.8360	5	23.8724	23.8384	7.28	6.8	9627.6
6	7.007	6.687	25.1181	5	25.1527	25.1196	6.92	6.62	9495.07
7	30.987	28.753	19.7268	5	19.8826	19.7381	31.16	28.9	40829.73
8	12.453	11.720	20.7091	5	20.7743	20.7134	13.04	12.18	16642.4
9	5.320	4.993	23.8369	5	23.8635	23.8384	5.32	5.02	7090.53
10	1.180	1.053	25.6764	5	25.6822	25.6770	1.16	1.04	1495.73
11	1.227	1.153	19.8409	5	19.8470	19.8412	1.22	1.16	1637.73
12	2.160	2.020	21.0917	5	21.1033	21.0920	2.32	2.26	2868.4
13	1.627	1.473	18.5468	5	18.5550	18.5476	1.64	1.48	2092.13
14	1.640	1.473	25.2074	5	25.2153	25.2082	1.58	1.42	2092.13
15	1.713	1.580	30.0614	5	30.0697	30.0621	1.66	1.52	2243.6
16	4.160	3.980	21.0913	5	21.1138	21.0922	4.5	4.32	5651.6
17	4.000	3.713	20.6701	5	20.6902	20.6717	4.02	3.7	5272.93
18	3.333	3.140	33.9542	5	33.9686	33.9551	2.88	2.7	4458.8
19	3.480	3.307	20.6526	5	20.6701	20.6536	3.5	3.3	4695.47

20	3.720	3.540	25.6770	5	25.6954	25.6777	3.68	3.54	5026.8
21	1.753	1.553	22.2360	5	22.2451	22.2368	1.82	1.66	2205.73
22	3.953	3.760	23.6391	5	23.6587	23.6402	3.92	3.7	5339.2
23	3.713	3.393	25.1184	5	25.1371	25.1199	3.74	3.44	4818.53
24	3.060	2.567	33.9532	5	33.9688	33.9556	3.12	2.64	3644.67
25	7.480	6.880	19.8159	5	19.8531	19.8187	7.44	6.88	9769.6
26	6.380	5.867	30.0617	5	30.0943	30.0644	6.52	5.98	8330.67
27	3.040	2.793	19.7271	5	19.7424	19.7283	3.06	2.82	3966.53
28	22.833	21.040	20.6962	5	20.8087	20.7046	22.5	20.82	29876.8
29	17.533	16.453	33.9543	5	34.0317	33.9573	15.48	14.88	23363.73
30	24.853	23.213	21.2277	5	21.3497	21.2358	24.4	22.78	32962.93
32	2.167	2.080	19.8399	5	19.8512	19.8403	2.26	2.18	2953.6
33	26.153	24.740	20.6721	5	20.8013	20.6793	25.84	24.4	35130.8
34	18.180	17.327	27.5976	5	27.6853	27.6016	17.54	16.74	24603.87
35	7.360	7.060	19.8110	5	19.8480	19.8123	7.4	7.14	10025.2
36	18.933	17.687	31.0952	5	31.1854	31.0997	18.04	17.14	25115.07
37	3.647	3.487	21.1642	5	21.1821	21.1648	3.58	3.46	4951.07
38	4.327	4.120	20.6536	5	20.6753	20.6543	4.34	4.2	5850.4
39	1.900	1.700	21.0922	5	21.1030	21.0935	2.16	1.9	2414

#5.2 – TSS and VSS data obtained from the analyses performed over samples from the CLAR (Replicate 2)

Sampling day	TSS (g/L)	VSS (g/L)	Replicate 2						COD of biomass (mg)
			net (g)	V (mL)	moven (g)	mmuffle (g)	TSS (g/L)	VSS (g/L)	
2	1.147	1.033	19.8410	5	19.8469	19.8418	1.18	1.02	1467.33
3	7.253	6.780	25.1179	5	25.1538	25.1201	7.18	6.74	9627.6
6	7.007	6.687	20.6539	5	20.6899	20.6558	7.2	6.82	9495.07
7	30.987	28.753	22.2349	5	22.3898	22.2462	30.98	28.72	40829.73
8	12.453	11.720	23.6397	5	23.6998	23.6428	12.02	11.4	16642.4
9	5.320	4.993	25.2079	5	25.2337	25.2095	5.16	4.84	7090.53
10	1.180	1.053	23.6394	5	23.6455	23.6400	1.22	1.1	1495.73
11	1.227	1.153	21.1613	5	21.1674	21.1617	1.22	1.14	1637.73
12	2.160	2.020	30.0615	5	30.0719	30.0625	2.08	1.88	2868.4
13	1.627	1.473	22.2353	5	22.2431	22.2360	1.56	1.42	2092.13
14	1.640	1.473	23.6372	5	23.6454	23.6379	1.64	1.5	2092.13
15	1.713	1.580	20.6530	5	20.6616	20.6533	1.72	1.66	2243.6
16	4.160	3.980	20.7094	5	20.7291	20.7102	3.94	3.78	5651.6
17	4.000	3.713	19.7271	5	19.7472	19.7286	4.02	3.72	5272.93
18	3.333	3.140	31.0971	5	31.1125	31.0981	3.08	2.88	4458.8
19	3.480	3.307	19.8174	5	19.8346	19.8182	3.44	3.28	4695.47
20	3.720	3.540	19.8410	5	19.8597	19.8421	3.74	3.52	5026.8

21	1.753	1.553	27.5968	5	27.6059	27.5985	1.82	1.48	2205.73
22	3.953	3.760	21.0934	5	21.1129	21.0934	3.9	3.9	5339.2
23	3.713	3.393	20.7087	5	20.7277	20.7108	3.8	3.38	4818.53
24	3.060	2.567	31.0966	5	31.1118	31.0977	3.04	2.82	3644.67
25	7.480	6.880	20.6535	5	20.6910	20.6562	7.5	6.96	9769.6
26	6.380	5.867	25.6776	5	25.7094	25.6801	6.36	5.86	8330.67
27	3.040	2.793	25.2087	5	25.2239	25.2099	3.04	2.8	3966.53
28	22.833	21.040	20.6722	5	20.7863	20.6810	22.82	21.06	29876.8
29	17.533	16.453	31.0976	5	31.1898	31.1039	18.44	17.18	23363.73
30	24.853	23.213	22.2362	5	22.3607	22.2442	24.9	23.3	32962.93
32	2.167	2.080	25.2073	5	25.2179	25.2077	2.12	2.04	2953.6
33	26.153	24.740	25.6776	5	25.8116	25.6849	26.8	25.34	35130.8
34	18.180	17.327	21.0925	5	21.1908	21.0973	19.66	18.7	24603.87
35	7.360	7.060	20.6535	5	20.6900	20.6549	7.3	7.02	10025.2
36	18.933	17.687	33.9525	5	34.0480	33.9598	19.1	17.64	25115.07
37	3.647	3.487	27.5988	5	27.6168	27.5994	3.6	3.48	4951.07
38	4.327	4.120	19.8413	5	19.8630	19.8426	4.34	4.08	5850.4
39	1.900	1.700	19.8098	5	19.8184	19.8105	1.72	1.58	2414

#5.3 – TSS and VSS data obtained from the analyses performed over samples from the CLAR (Replicate 3)

Sampling day	TSS (g/L)	VSS (g/L)	Replicate 3						COD of biomass (mg)
			net (g)	V (mL)	moven (g)	mmuffle (g)	TSS (g/L)	VSS (g/L)	
2	1.147	1.033	19.0651	5	19.0707	19.0654	1.12	1.06	1467.33
3	7.253	6.780	19.8158	5	19.8523	19.8183	7.3	6.8	9627.6
6	7.007	6.687	19.8419	5	19.8764	19.8433	6.9	6.62	9495.07
7	30.987	28.753	21.1622	5	21.3163	21.1731	30.82	28.64	40829.73
8	12.453	11.720	20.6709	5	20.7324	20.6745	12.3	11.58	16642.4
9	5.320	4.993	18.5469	5	18.5743	18.5487	5.48	5.12	7090.53
10	1.180	1.053	22.2358	5	22.2416	22.2365	1.16	1.02	1495.73
11	1.227	1.153	20.7087	5	20.7149	20.7091	1.24	1.16	1637.73
12	2.160	2.020	19.7273	5	19.7377	19.7281	2.08	1.92	2868.4
13	1.627	1.473	20.6935	5	20.7019	20.6943	1.68	1.52	2092.13
14	1.640	1.473	21.2262	5	21.2347	21.2272	1.7	1.5	2092.13
15	1.713	1.580	19.0645	5	19.0733	19.0655	1.76	1.56	2243.6
16	4.160	3.980	19.8413	5	19.8615	19.8423	4.04	3.84	5651.6
17	4.000	3.713	27.5969	5	27.6167	27.5981	3.96	3.72	5272.93
18	3.333	3.140	31.5853	5	31.6003	31.5861	4.04	3.84	4458.8
19	3.480	3.307	21.1624	5	21.1799	21.1632	3.5	3.34	4695.47
20	3.720	3.540	20.6715	5	20.6902	20.6724	3.74	3.56	5026.8

21	1.753	1.553	18.5465	5	18.5546	18.5470	1.62	1.52	2205.73
22	3.953	3.760	21.2276	5	21.2478	21.2294	4.04	3.68	5339.2
23	3.713	3.393	19.0657	5	19.0837	19.0669	3.6	3.36	4818.53
24	3.060	2.567	31.5861	5	31.6012	31.5900	3.02	2.24	3644.67
25	7.480	6.880	19.8093	5	19.8468	19.8128	7.5	6.8	9769.6
26	6.380	5.867	23.8378	5	23.8691	23.8403	6.26	5.76	8330.67
27	3.040	2.793	19.8406	5	19.8557	19.8419	3.02	2.76	3966.53
28	22.833	21.040	21.1627	5	21.2786	21.1724	23.18	21.24	29876.8
29	17.533	16.453	31.5862	5	31.6796	31.5931	18.68	17.3	23363.73
30	24.853	23.213	20.7098	5	20.8361	20.7183	25.26	23.56	32962.93
32	2.167	2.080	20.6964	5	20.7070	20.6969	2.12	2.02	2953.6
33	26.153	24.740	21.1645	5	21.2936	21.1712	25.82	24.48	35130.8
34	18.180	17.327	19.0655	5	19.1522	19.0695	17.34	16.54	24603.87
35	7.360	7.060	23.6394	5	23.6763	23.6412	7.38	7.02	10025.2
36	18.933	17.687	31.5864	5	31.6847	31.5933	19.66	18.28	25115.07
37	3.647	3.487	21.2285	5	21.2473	21.2297	3.76	3.52	4951.07
38	4.327	4.120	20.7099	5	20.7314	20.7110	4.3	4.08	5850.4
39	1.900	1.700	20.6709	5	20.6800	20.6719	1.82	1.62	2414